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A STUDY OF SAPONIN HEMOLYSIS OF INDIVIDUAL ERYTHROCYTES

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The assumption has been made by numerous workers that red blood corpuscles vary in resistance towards various hemolytic agents, and that this variation is distributed approximately according to a bell-shaped probability curve. Smith (1) found the percentage hemolysis of horse erythrocytes to follow a sigmoid curve, and from this arranged the cells according to their resistance in a frequency curve which was more or less symmetrically bell-shaped. Arrhenius (2) came to a similar conclusion on studying the hemolysis of erythrocytes by vibriolysin. Brooks (3), studying hemolysis by ultra-violet radiation and by serum, believed that the course of hemolysis is largely dependent upon variation in resistance among the erythrocytes. Ponder (4) concluded from the sigmoid time curves of various types of hemolysis, including that by saponin, that the erythrocytes fall into a symmetrical frequency distribution according to their resistance.

Previous work (5) led us to question this probability distribution. Diminishing cell concentration, the effects of one cell upon its neighbors, and the effects of the products of hemolysis upon cells still unhemolyzed, are among the factors which undoubtedly influence the shape of the hemolysis curve. It was the object of this study to so alter the cell environment as to obviate all these effects.

The fundamental principle was to isolate a single red blood cell in a small droplet of saponin solution, and to observe it microscopically until it hemolyzed, noting the time required. By means of a sufficiently large series of such individual observations, a composite picture was obtained of

[1] Cutting Travelling Fellow, Columbia University.

the process of saponin hemolysis in which all influence of one cell upon another, and of products of hemolysis upon the hemolysis of subsequently hemolyzing cells, was obviated.

TECHNIQUE. One-hundredth cubic centimeter of blood was taken from a finger-prick, suspended in about 15 cc. of modified Brinkman's solution (6), and centrifuged slowly. The cells were resuspended in 25 cc. of modified Brinkman's solution, and approximately 1.0 cc. of this suspension was diluted in 50 cc. of modified Brinkman's solution. This suspension was allowed to equilibrate (5) 18 to 20 hours at 2 to 4°C., in a paraffined flask. Shortly before using, the suspension was warmed to the same room temperature used in previous work, approximately 22°C. One cubic centimeter was placed in a small test-tube. One cubic centimeter of a saponin solution of twice the concentration to be studied was then added rapidly from a pipette, and the mixture agitated gently until the total time elapsed since the beginning of the addition of saponin was 20 seconds. By means of a capillary pipette (of a bore such that a 10 cm. length of fluid column measured approximately 0.006 cc.) several droplets, each having a volume of about 0.0001 cc., were rapidly placed on the carefully cleaned, decharged (7), glass stage of a closed moist chamber, and immediately observed microscopically. By using a mono-objective, binocular microscope, it was possible to quickly detect the presence or absence of one or more red cells in a droplet under a magnification of eighty. Whichever droplet contained only one red cell was observed until the cell had hemolyzed, and the elapsed time from the addition of saponin to hemolysis was noted. The end-point, complete disappearance of the red cell, was regularly sharp and definite. For a few seconds before the cell was due to hemolyze, it seemed to increase slightly in diameter and to become somewhat less refractive. It then faded away rapidly without further change in form, and within a period of about ten seconds no vestige of it could be detected. Heating effects were obviated and visibility of the red cells increased by using diffuse sky light as the source of illumination, with the microscope condenser lowered and the diaphragm at its minimum opening.

The above procedure was repeated from one hundred to several hundred times, depending upon the particular experiment, and the observations of each experiment combined to yield a single curve. Inasmuch as the hemolysis of a single red cell consumed from one to more than thirty minutes, a single experiment involving one hundred or more such observations required from one to several weeks. Accordingly, conditions were standardized as far as possible. The same human source of blood was used throughout. Blood was taken at the same time of day on successive days. Chemicals and saponin were identical throughout, except as noted.

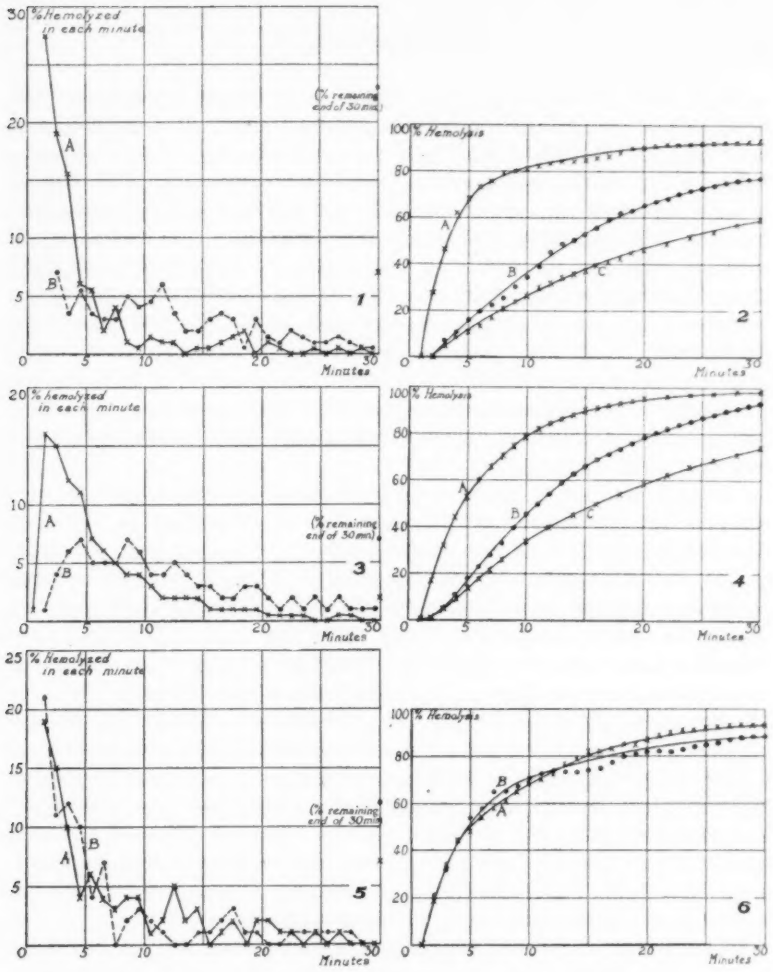

Fig. 1. Saponin hemolysis of individual erythrocytes. Ordinates represent percentage of total series which hemolyzed in each minute. Abscissae equal minutes after addition of saponin. Curve *A* — saponin 1:30,000. Curve *B* — saponin 1:35,000.

Fig. 2. Hemolysis "time" curves obtained by integrating curves of figure 1. Curve *A* — saponin 1:30,000; *B* — 1:35,000; *C* — 1:40,000.

Fig. 3. Saponin hemolysis, 30 to 70 cells per drop. Coördinates same as figure 1 Curve *A* — saponin 1:30,000; *B* — 1:35,000.

Fig. 4. Hemolysis "time" curves obtained by integrating curves of figure 3. *A* — saponin 1:30,000; *B* — 1:35,000; *C* — 1:40,000.

Fig. 5. Saponin hemolysis of individual erythrocytes, comparing two methods of mixing saponin and cell suspension. Curve *A* — saponin added during 2 seconds, shaken 8 seconds. *B* — added 20 seconds, shaken 10 seconds. Saponin 1:35,000. Coördinates same as figure 1.

Fig. 6. Hemolysis "time" curves obtained by integrating curves of figure 5. *A* — 2 + 8 seconds. *B* — 20 + 10 seconds.

EXPERIMENTAL OBSERVATIONS. Curve *A* of figure 1 represents the hemolysis of two hundred corpuscles observed singly in a saponin concentration of 1:30,000. Abscissae represent elapsed time in minutes following the addition of saponin solution to cell suspension. The number of cells, expressed as per cent, which hemolyzed in each one minute time interval, is plotted as an ordinate at the mid-point of the corresponding time interval, and the points so determined are connected by straight lines. During the first minute following mixing no cells were observed to hemolyze. During the second minute 55 cells or 27.5 per cent hemolyzed, following which the rate of hemolysis became progressively slower until only an occasional cell hemolyzed after twenty minutes had elapsed. Observations were discontinued at the end of thirty minutes. The percentage of cells remaining unhemolyzed at that time is indicated on the corresponding ordinate.

Curve *B* of figure 1 represents the results of a similar series of two hundred determinations, but in a saponin concentration of 1:35,000. Accordingly hemolysis is less vigorous, but of the same general character as in 1:30,000 saponin. No cells hemolyzed during the first two minutes.

Curve *A* of figure 2 is the integral of curve *A* of figure 1, obtained by summation, and is comparable to the usual time curve of hemolysis obtained by determining per cent of hemolysis of a cell suspension at intervals and plotting it against time. Curve *B* of figure 2 is the integral of curve *B* of figure 1, using saponin in 1:35,000 concentration. Curve *C* of figure 2 represents the integrated results of a third series of determinations in a saponin concentration of 1:40,000 (omitted from fig. 1 to avoid confusion of lines). The most striking feature of these three curves is that, if one may look upon them for purposes of analysis as time curves of a kinetic reaction, the reaction exhibits a maximum rate of hemolysis at the beginning, with a progressive diminution thereafter. This is in sharp contrast to the usual sigmoid time curve of saponin hemolysis.

When, however, the cell concentration is increased so that a droplet contains from thirty to seventy red blood cells, a different type of reaction curve is obtained. Figure 3 is analogous to figure 1. Curve *A* (fig. 3) represents the hemolysis in 1:30,000 saponin of over a thousand red cells handled exactly as above except that the final 1:50 dilution was omitted. (To make the final plasma concentration the same as in the single cell experiments, the cells were washed twice by centrifuging.) Each droplet contained, accordingly, from thirty to seventy cells, and the time required for the hemolysis of each cell was noted. It will be observed that the maximum number of cells does not hemolyze in the first time period following the latent period, but in the second, in the case of 1:30,000 saponin. Using 1:35,000 saponin (curve *B*, fig. 3) maximum hemolysis is still further delayed.

Curve *A* of figure 4 is the integral of the data of curve *A*, figure 3, and shows a faint tendency to be sigmoid. When the saponin concentration is reduced to 1:35,000 or 1:40,000 (curves *B* and *C*, fig. 4), the maximum number of cells hemolyze during a later time period and the integral curves become more definitely sigmoid. Were it possible to follow visually the hemolysis of each cell in droplets containing more than 30 to 70 cells, it is possible that a more symmetrical sigmoid curve might be obtained.

The question arises as to what effect the method and rate of mixing of the saponin solution with the cell suspension might have on the reaction. In the determinations reported above, 1 cc. of a given saponin solution was rapidly run into 1 cc. of red cell suspension from a pipette of large orifice so that about three seconds were consumed in the addition. Mixing by gentle agitation was carried on until 20 seconds had elapsed from the beginning of the process. It seemed possible that the time required to hemolyze a cell might be wholly or partially dependent upon the rate at which saponin was made available to the cell. To throw light on this, two parallel series of determinations, each of one hundred individual cell observations, were carried on simultaneously. Each determination of one series alternated with a determination in the other, the same solutions being used in both. In one series 1 cc. of saponin solution was added during 2 seconds from a pipette of large orifice to 1 cc. of cell suspension, following which the mixture was gently agitated during 8 seconds until a total of 10 seconds had elapsed from the beginning of addition of saponin. Droplets were then removed and observed as usual. Inasmuch as the reverse process, addition of cell suspension to saponin, was impracticable because it would expose the initial cells to a saponin concentration twice that of the desired concentration, the second series consisted in adding 1 cc. of saponin solution during 20 seconds from a pipette of small orifice to 1 cc. of cell suspension, followed by gentle mixing during 10 seconds until a total of 30 seconds had elapsed. The final saponin concentration was 1:35,000. Figure 5 gives the results of these two series, the number of cells observed to hemolyze during any given minute being plotted as ordinate in the mid-point of the corresponding time interval as abscissa.[2] Both curves have the same general form and dimensions. The similarity is brought out further in the corresponding integral curves of figure 6. Accordingly the difference between these two methods of mixing does

[2] The chemicals used in making up the modified Brinkman's solution used in these two series were unavoidably of different manufacture from those used in the other determinations. Furthermore, the determinations of these two series were made some months after the others so that the blood, though from the same individual and of the same red cell count, may have been somewhat different. These changes probably account for the apparent increase in hemolytic activity of the saponin at the concentration used, in the later determinations

not materially influence the general reaction. Furthermore these two curves are of the same type as those of the previous individual cell determinations.

DISCUSSION. When the hemolysis of a sufficiently large series of red cells is observed, each cell hemolyzing apart from all influence of other cells, one finds, not that all the cells hemolyze at the same time, which one might expect if all the cells were alike, but that some cells hemolyze more readily than others, the greatest number hemolyzing during the period immediately following the addition of the hemolytic agent. If this is a manifestation of individual cell resistance, such resistance is distributed not according to a bell-shaped or to a skewed probability curve, but approximately according to one lateral half of such a curve. This is a concept totally different from those usually accepted, and one which would make necessary a reconsideration of many of the observations which have been made on various types of hemolysis. Such a distribution, furthermore, is quite unusual in biological statistics. The results can be more logically explained by assuming that all the cells are alike and identical, analogous to chemical molecules, and that the hemolysis of any given cell depends upon the completion of some reaction or set of reactions which goes on according to the laws of chance.[3] Yule (8) and Robertson (9) have already formulated a similar conception as applied to the death-rate of bacteria. According to this view, any given erythrocyte hemolyzes when a certain reaction completes itself in that cell. Completion of the reaction depends upon the fortuitous collisions of saponin molecules with the erythrocyte. The shape of the curve representing the hemolysis of a series of cells is an expression of this fortuity.

SUMMARY

A method is described for the study of saponin hemolysis of single, isolated, red blood cells.

Utilizing this method, several series of determinations of single cell hemolysis were carried out. In each series the greatest number of cells was found to hemolyze during the earlier time intervals after addition of saponin, with a progressive falling off in number of cells hemolyzed as the time increased. This curve is distinctly different from the derivative of the usual sigmoid hemolysis time curve, and since observations on individual cells should, if anything, accentuate the probability distribution of individual cell resistances, no evidence can be found for such a distribution. It seems less probable that the curve is a manifestation of individual cell

[3] This explanation is that of Prof. E. B. Phelps of the Department of Sanitary Science, Columbia University, to whom we are very greatly indebted for this and other valuable suggestions.

resistance than of the fortuitous completion of the hemolytic reaction on identical erythrocytes.

It is a pleasure to express our appreciation and thanks to Prof. C. Prausnitz and Dr. H. Lubinski of the Institute of Hygiene, Breslau, for their kind hospitality and aid in making possible the greater part of this work. Dr. T. F. Zucker of the Department of Pathology, College of Physicians and Surgeons, has kindly given many suggestions and helpful criticisms.

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EVIDENCE OF FUNCTIONAL RÔLE OF BASILAR MEMBRANE IN AUDITION

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Objection (1) has been made to Helmholtz's (2) second theory of audition on the basis of the presence of the cochlear artery in the basal membrane of the organ of Corti. So far as I know, however, no physiological evidence has ever been offered to support this objection. It has been stated (1) that vasomotor effects on this large vessel would interfere with the hypothetical function of the basement membrane, but this has never been subjected to experimental observation. The experiment should be readily performable as vasomotor drugs are available and are relatively harmless to administer; furthermore, due to the peculiar distribution of the artery, direct vasomotor effects can be easily separated from indirect ones. The sole arterial supply to the internal ear is through a single vessel which enters the labyrinth by way of the internal acoustic meatus (3). This vessel gives off the cochlear artery, the main single branch of which lies under the membrana basilaris at that portion of the organ of Corti which detects tones between approximately 1024 and 8192 double vibrations. The more basal portions of the cochlea, which receive the higher tones, are supplied not by this single vessel but by numerous small ones. The same applies to portions of the cochlea apical to this region, where the lower tones are heard. If vasomotor changes affect audition it should be that part of the tone scale received by the organ of Corti supplied by this large single artery.

METHODS. The general blood pressure was altered by the use of sodium nitrite and epinephrine. Sodium nitrite in three to five grain doses was given by mouth. Epinephrine in doses of from fifteen to thirty minims was given hypodermically and massaged (4) to get the full effect. Within twenty to thirty minutes after nitrite administration there was a fall in blood pressure of from 20 mm. Hg to 40 mm. Hg. The maximal blood pressure effect persisted for from $1\frac{1}{2}$ to $2\frac{1}{2}$ hours. The maximum rise in blood pressure from subcutaneous epinephrine injection occurred in from 10 to 20 minutes and persisted from $1\frac{1}{2}$ to 2 hours, but could be maintained at will by massaging the area injected.

These drugs affect blood pressure, of course, mostly by their vasomotor action. The nitrite vaso-dilatation and the epinephrine vaso-constriction may be seen in the retinal vessels of man and of animals ophthalmoscopically. It is reasonable to infer by analogy that the cochlear arteries dilate and contract as do the retinal arteries under the action of these drugs. Nevertheless, the action may be different. Whatever the vasomotor nerve supply, the nitrites would act by vasodilatation because of their direct action upon the vessel musculature without action upon the nerves. The facts observed, and recorded herein later, demonstrate that the two drugs alter audition distinctly and in *opposite* directions. Therefore, since we know that nitrite produces vasodilatation of all medium and small arteries, it is practically certain that epinephrine produces vasoconstriction of the cochlear artery.

Auditory acuity was determined by use of the audiometer. (Western Electric Model I A.)

Results. Blood pressure variations produced alterations in audition *only in one portion of the tone scale.* The alteration occurred only between the limits of 512 and 12,228 double vibrations. Usually the alteration was confined to the limits of 1024 to 8192. In some of the individuals tested the limit was extended half an octave above this point and in other individuals the lower limit was an octave below, that is, at 512. It is assumed that such variation was due to a corresponding variation in the points at which the large cochlear artery divided into its smaller branches.

A rise in blood pressure of even so small a degree as 5 mm. Hg to 10 mm. Hg produced a distinct increase in auditory acuity between the limits specified (fig. 1).

Greater increases in blood pressure produce more marked increase in auditory acuity but the two are not proportional. As the blood pressure increase becomes greater the auditory acuity increases less and less (fig. 2).

It was interesting to observe that patients with moderate distortion of their "normal" audiograms experienced similar increase in auditory acuity with experimental blood pressure increases (fig. 3).

Lowering the blood pressure with sodium nitrite produced even more marked variations in auditory acuity. The auditory acuity was decreased and the decrease was within exactly the same limits as observed for the increased acuity produced by elevation of the blood pressure (figs. 4, 5).

Discussion. The evidence presented here is too limited in its scope to warrant the consideration of a new theory of audition. The application of this evidence to the major theories will, perhaps, not be amiss.

It is certain that the relatively large cochlear artery running along the under side of the basal portions of the Corti tunnel does *not* render the Helmholtz second theory of audition untenable. Shambaugh (1), on the basis of purely anatomical observation and deduction, has stated: "If the

membrane (basilar) should not respond equally at all times to the same impulse, there could be, for example, no basis for the recognition of pitch. As I (5) have previously pointed out, there exists an anatomical condition which makes such a response a physical impossibility, and which, therefore, constitutes an unanswerable argument against any theory which placed the active rôle in the membrana basilaris. This anatomical condition is the presence of the blood vessel attached throughout the cochlea to the under surface of the membrana basilaris. This blood vessel, I have been able to demonstrate by injection experiments, is capable of the same distention and contraction, depending on varying degrees of blood pressure, as are the blood vessels in other parts of the body."

Thus practically the only argument advanced in favor of the tectorial theory is that the basilar membrane is supposably theoretically unsuitable. However, now that we know that it is a fact that the basilar membrane is not only suitable but that it is essentially concerned in audition, little remains even in argument favoring the tectorial theory. Dilatation of the cochlear artery by the use of nitrites causes an impairment of audition for that portion of the tone scale subserved by the organ of Corti directly over the unbranched artery. The effect of the nitrites is not central because there would be alteration of the entire tone scale such as occurs from gastro-intestinal stimulation (6) or from the use of caffeine (fig. 6), in both of which types of stimulation (*i.e.*, reflex and c. n. s. drug stimulation) the increase of auditory acuity is throughout the entire tone scale and is not restricted to any portion of it.

If, therefore, vasomotor changes in this artery occur, as they must with nitrites and most probably do with epinephrine, and as Shambaugh (1)

Fig. 1. Audiogram showing increase of auditory acuity produced by raising the blood pressure from a normal of 125 mm. Hg to 135 mm. Hg. Figures to left of graph indicate sensation units.

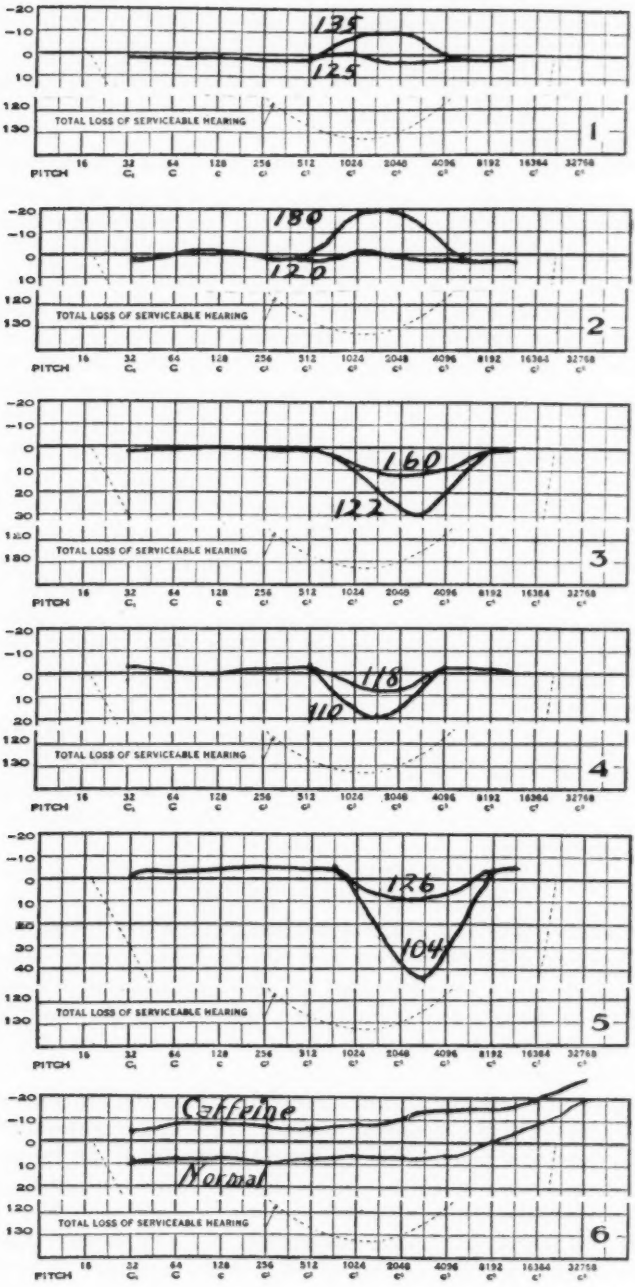
Fig. 2. Audiogram showing marked increase in auditory acuity produced by raising blood pressure from normal of 120 mm. Hg to 180 mm. Hg. Figures to left of chart indicate sensation units.

Fig. 3. Audiogram showing increased auditory acuity produced by raising blood pressure from normal of 122 mm. Hg to 160 mm. Hg. Note the considerable increase despite the depression of the "normal."

Fig. 4. Audiogram showing diminution of auditory acuity produced lowering blood pressure 8 mm. Hg. Normal, 118 mm. Hg. Lowered pressure, 110 mm. Hg.

Fig. 5. Audiogram showing marked diminution of auditory acuity produced by lowering blood pressure from normal of 126 mm. Hg to 104 mm. Hg. Note slight difference in portions of tone scale involved in audiograms 4 and 5 They are from different individuals.

Fig. 6. Audiogram showing the increase of auditory acuity involving the entire tone scale which occurred ½ hour after drinking a cup of coffee. The hyperacusis in the upper tones may be accounted for by the fact that this subject is a violinist. The upper line at 32,768 double variations is at −30 sensation units.


135
125
TOTAL LOSS OF SERVICEABLE HEARING
1
PITCH
180
120
TOTAL LOSS OF SERVICEABLE HEARING
2
PITCH
160
122
TOTAL LOSS OF SERVICEABLE HEARING
3
PITCH
118
110
TOTAL LOSS OF SERVICEABLE HEARING
4
PITCH
126
104
TOTAL LOSS OF SERVICEABLE HEARING
5
PITCH
Cakfeine
Normal
TOTAL LOSS OF SERVICEABLE HEARING
6
PITCH

Figs. 1 to 6

states they do, and they alter audition to as much as 30 per cent, then surely there is no escaping the conclusion that the membrana basilaris is actively essential in audition.

The theory that the basilar membrane vibrates as a whole (Waller, Meyer, ter Kuile, Ewald) is untenable if the data presented herein have been interpreted correctly. It is inconceivable that weighting or damping a considerable portion of a vibrating membrane would markedly depress the vibrations at the damped portion and not affect the other portions. The cochlear artery is large and central in only one portion of the basilar membrane. Damping it by vasodilatation at this portion affects audition for this portion of the cochlea only. If certain tones and their harmonics were affected by the damping process one could consider that the basilar membrane vibrated as a whole. But to be able to damp one portion without modifying any other portion is to render completely untenable the theory.

The tectorial theory as proposed by Rutherford (9) is unable to explain the experimental data presented. If the tectorial membrane and hair cells from base to apex of the cochlea act like the membrane and magnets of a telephone transmitter, how is one to account for this damping effect? If a telephone transmitter is out of adjustment it is easy to imagine that the entire tone scale or either end of it might suffer thereby, but how could a limited middle portion be affected, leaving the low and highest tones unaltered?

Therefore, it is concluded that tone analysis must be peripheral, that the Ewald et al. theories are untenable and that to adequately explain these facts it is necessary to assume that local segments of the basilar membrane have essential functional rôles in audition. It is not denied that the tectorial membrane may also be essential.

SUMMARY AND CONCLUSIONS

1. A number of normal adults were given 2 grams to 4 grams of sodium nitrite to lower the blood pressure and the alterations in their audiograms were studied. It is considered that the alteration was due to vasodilatation of the cochlear artery in its unbranched portions, the vasodilatation producing increased weight to the basilar membrane and interfering with its auditory function.

2. Fifteen to thirty minims of epinephrine were administered to these individuals and their audiograms studied. Reasons are given for assuming that vasoconstriction of the cochlear artery results, the vasoconstriction decreasing the weight of the basilar membrane and increasing its auditory function.

3. It was found that marked depression and considerable increase of auditory acuity resulted in that portion of the organ of Corti directly

over the unbranched cochlear artery which responded by vasodilatation and vasoconstriction.

4. It is concluded that the basilar membrane is actively concerned in audition although it is not denied that the tectorial membrane may also be actively necessary.

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PHYSIOLOGY OF THE CORPUS LUTEUM

IV. Production of Artificial Deciduomata with Extracts of the Corpus Luteum[1]

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In 1907 and 1908 Loeb published a series of experiments in which he produced deciduomata by indifferent stimulation of the uterine mucosa at certain periods of the sexual cycle. By incising the endometrium at various places at approximately 2 to 9 days after ovulation, he obtained within 10 days after the operation as many maternal placentas as cuts had been made. These artificially produced deciduomata were structurally identical with the normal maternal placenta developed during pregnancy. However, removal of the ovaries previous to incision of the uterus prevented the development of deciduomata.

Loeb concluded that the formation of a deciduoma at the site of trauma is dependent upon two factors: first, the chemical action of a sensitizing substance secreted by the ovaries which enters the circulation and evidently fixes itself upon the uterine mucosa, and second, the action of certain indifferent stimuli on the sensitized mucosa, such as an incision into the endometrium, the introduction of a foreign body into the lumen of the uterus, or the drawing of a suture through the uterine wall. From his results he also concluded that the stimulation of the endometrium by the ovum is comparable to such indifferent stimulation and that the specific effect is the sensitizing action of the ovarian substance on the uterine mucosa.

In 1903 and 1910 L. Frankel showed in a series of experiments on rabbits that the corpora lutea have an important function in implantation and maintenance of embryos during pregnancy. He found that removal of the ovaries or cauterization of the corpora lutea prevented implantation and normal development of the embryos. In 1910 Bouin and Ancel supported Frankel's results by demonstrating characteristic changes in the

[1] The first, second, and third papers of this series are those quoted in the bibliography as Corner, 1928; Corner and Allen, 1929; Allen and Corner, 1929.

rabbit's uterus simultaneous with the development of the corpora lutea. This process of endometrial proliferation was prevented by extirpation or cauterization of the corpora lutea. In 1928 Corner confirmed the existence of progestational proliferation of the rabbit's uterus during the early days of pregnancy and the dependence of such proliferation upon the corpora lutea. His experiments also confirmed Frankel's view that the corpora lutea are essential for implantation of the embryos. He suggested that normal implantation in the rabbit and proper nutrition of the embryos are dependent upon progestational proliferation of the uterine mucosa.

Corner and Allen (1929) found that by injection of their alcoholic extracts of corpus luteum (prepared as described in the second paper of this series) into rabbits, öophorectomized shortly after ovulation, they could produce progestational proliferation identical with that normally produced in the presence of corpora lutea. These investigators have been able to produce normal implantation and growth of the embryos to the 13th and 18th days in ovariectomized rabbits treated with their corpus luteum extract. In recent unpublished experiments they have also obtained implantation and normal development of the embryos throughout the course of gestation, and spontaneous delivery at term under the same experimental conditions. This extract apparently contains a special hormone necessary to the preparation of the uterus for implantation and maintenance of the embryos.

Weichert (1928), working with Hisaw, found that if into guinea pigs which had been ovariectomized on the 4th day after oestrus and in which the uterine mucosa had been stimulated, corpus luteum hormone was injected at the time of the operation and continued for four days, deciduomata were formed. In control experiments in which no corpus luteum hormone was administered to ovariectomized guinea pigs, autopsy four days later showed that no deciduomata had been formed. These control experiments confirmed the previous work of Loeb.

Loeb and Kountz (1928) obtained negative results when using follicular extract instead of the corpus luteum extract.

Hisaw (1927) further observed a relaxation of the pubic ligaments in virgin guinea pigs injected shortly after oestrus with a serum prepared from the blood of pregnant guinea pigs, rabbits, cats, dogs and mares, or extracts of rabbit placentas and of the corpora lutea of the sow. He found that castrated female guinea pigs did not respond to the injection of this pregnant serum, but, if brought into artificial oestrus and then injected, relaxation of the symphysis pubis occurred as in normal pregnant guinea pigs.

At the suggestion of Prof. George W. Corner we have undertaken to test the extract prepared by Allen and Corner to see whether it will also produce deciduomata.

Our first series of experiments was performed on two virgin guinea pigs as a control of Loeb's original work and as an assurance of our technique. Five days after the animal had been in oestrus, an operation was performed under ether anesthesia and with aseptic precautions. The right cornu of the uterus was exposed by a flank incision. In each case the upper part of the cornu was incised and a silk suture drawn through the uterine wall below the incision. Care was taken to cut through the endometrium. At the same time we examined the right ovary and found freshly ruptured follicles. The animals were autopsied on the fifth and sixth days respectively after the operation. The entire reproductive tract was removed, fixed in Bouin's fluid, and paraffin sections were prepared for histological study.

On autopsy gross examination of the uterus showed at the site of trauma a large hyperemic tumor or growth which resembled a normal implantation. The pubic ligaments were slightly relaxed, as observed by Hisaw (1927). Microscopic study of these growths showed hyperemic decidual tissue characterized by a few scattered polynuclear giant cells, and mitotic figures.

Our results confirm the work of Loeb in producing maternal placentas by incision of the endometrium. However, it is interesting to note that drawing a silk suture through the uterine mucosa did not cause a growth. Perhaps this was due to the fact that the fine silk thread used did not produce sufficient trauma. In some cases, the growth caused by the incision enclosed the suture as in figure 1.

We used seven guinea pigs in a second series of experiments to test the corpus luteum extract of Corner and Allen. Virgin guinea pigs, it will be noted, were used in all our work. The technique in this series was the same as employed in the previous one. In addition both ovaries were excised by two flank incisions at the time of stimulation of one cornu of the uterus. The ovarian pedicles were clamped with a hemostat, the vessels ligated securely below the hemostat and the ovaries cut away. Precautions were taken to remove all the ovarian tissue. Examination of the excised ovaries at the time of the removal showed in each case, not only in this series of experiments but also in the succeeding series, freshly

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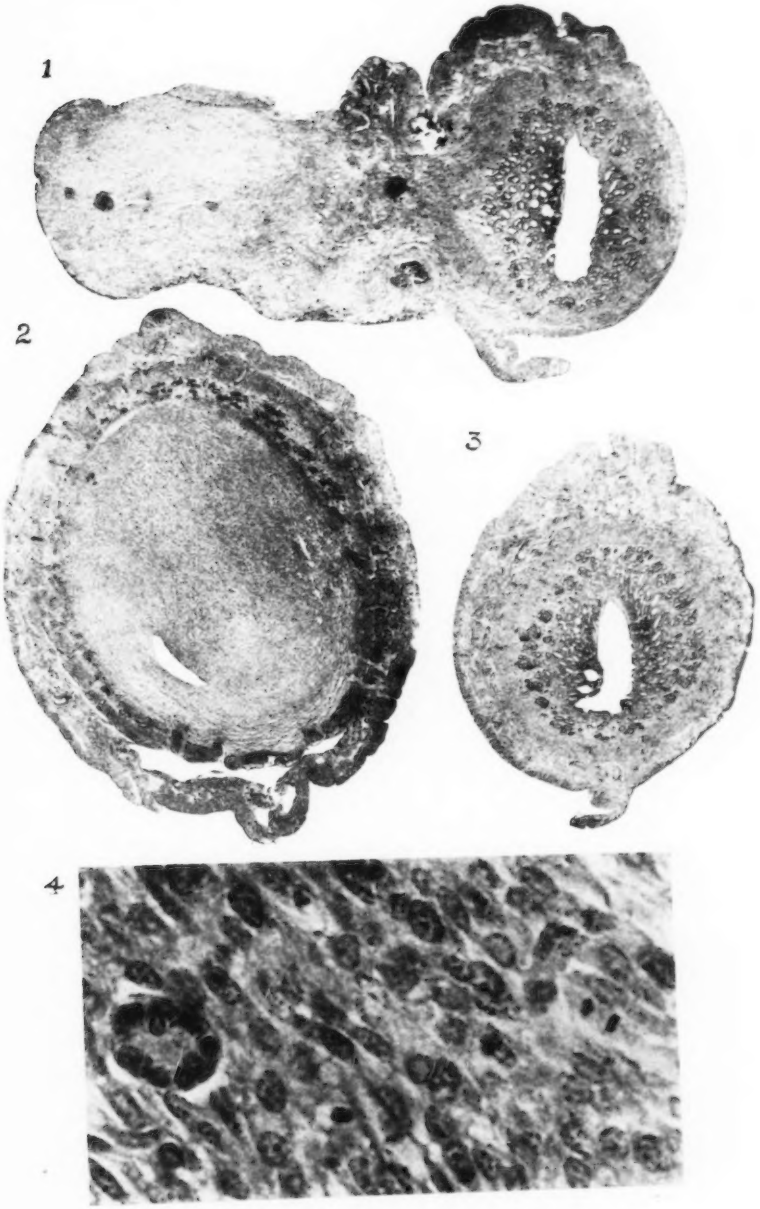
Fig. 1. Uterus of guinea pig 247, demonstrating external deciduoma at site of incision after 5 days' treatment with corpus luteum extract. Compare with figure 3. (× 18.)

Fig. 2. Uterus of guinea pig 246, demonstrating internal deciduoma at site of incision, after 5 days' treatment with corpus luteum extract. (× 18.)

Fig. 3. Control section of uterus of guinea pig 247, from non-traumatized cornu. (× 18.)

Fig. 4. Magnified view of section of deciduoma of guinea pig 247 (compare fig. 1) showing one polynuclear giant cell and a mitotic figure.



Figs. 1 to 4

ruptured follicles from two to eight in number. Five-tenths of a cubic centimeter of corpus luteum extract was injected subcutaneously immediately after the operation and at 24-hour intervals for four successive days. The animals were autopsied on the sixth day after operation. The ovarian stumps were examined under a binocular microscope and in no case was ovarian tissue found. The entire reproductive tract was removed and fixed for histological study. In each case the control preparation was taken from the cornu of the uterus which had not been stimulated. (See figs. 1, 2 and 3.)

Gross and microscopic examination of the tumors revealed results identical with those obtained with the ovaries intact (see fig. 4). The work further confirms the experiments of Hisaw and Weichert, and confirms the view that there is present in the corpus luteum extract used a special hormone which sensitizes the endometrium in the absence of corpora lutea.

A third series of experiments of three animals was run as a control set. The procedure was precisely the same as that used in the second series. Subcutaneous injections of estrogen (25 rat units per cc.) as prepared by Parke, Davis & Co. and mazola oil were substituted for injections of corpus luteum extract; 12.5 rat units of estrogen were injected daily into two guinea pigs for five successive days as in series II, and 0.5 cc. of mazola oil daily into the third guinea pig. In each case the results were negative. This confirms the work of Loeb and Kountz (1928).

CONCLUSIONS

Our experiments confirm the observation that corpus luteum extract contains a special hormone which has for one of its functions the sensitization of the uterine mucosa of the guinea pig so that deciduomata are formed upon indifferent stimulation of the endometrium during the proper period of the sexual cycle.

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OBSERVATIONS ON PERIPHERAL INHIBITION IN ARTHROPODS

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In vertebrates all inhibition of skeletal muscle has its seat of action in the central nervous system. Therefore the study of inhibition is complicated by the intricacy of central nervous organization. In certain invertebrates the conditions are simpler. Richet (1880) first showed that stimulation of the nerve to the claw of the crayfish caused opening or closing depending on the strength of stimulus applied. Luchsinger (1882) and later Biederman (1887) studied the phenomenon and proved that it involves a true reciprocal inhibition, the contraction of the adductor or closing muscle of the claw being accompanied by relaxation of its antagonist, the abductor or opener muscle, and vice versa. Biederman further described a positive variation of the galvanometer connected with the inhibited muscle. He found evidence for a dual nerve supply to each muscle element. According to Biederman's hypothesis, there are at least two kinds of nerve fibers, excitatory and inhibitory, in the nerve to the claw muscles. The fibers causing contraction of the abductor may branch and send inhibitory fibers to the adductor. Other fibers have the opposite effect. Piotrowski (1893) and Fröhlich (1907) have confirmed most of Biederman's conclusions. Additional support is found in the later work of Mangold (1905) whose histological studies confirmed the termination of paired fibers in each muscle fiber, an arrangement he found to be general in arthropods.

It seemed worth while to investigate this type of inhibition further with the hope that it might throw new light on the general problem. Our experiments were carried out on the lobster (Homarus), the blue crab (Callinectis), and the spider crab (Libinia), forms readily obtained at the Woods Hole laboratory. One of the large claw-bearing appendages was removed and a length of nerve sufficient for stimulation was dissected out. The claw was fixed in a suitable clamp and the adductor and abductor muscles connected to separate levers. Either ordinary light muscle levers or torsion-wire levers of the type described by Fulton (1925) were used.

Records were thus either isotonic or isometric. It should be noted that the dissection of the nerve must be done with the greatest care. Biederman noted that the isolated nerve of the crayfish was inactive. In the larger crabs used by us it is possible to make an active preparation if freshly caught material is used and great care is taken to avoid traction on the nerve. The excitatory fibers to the abductor are the most sensitive and quickly lose their irritability unless handled with the greatest care. For stimulation, we employed either the Harvard inductorium, condenser discharge; or the vacuum tube stimulator, described by Campbell (1929).

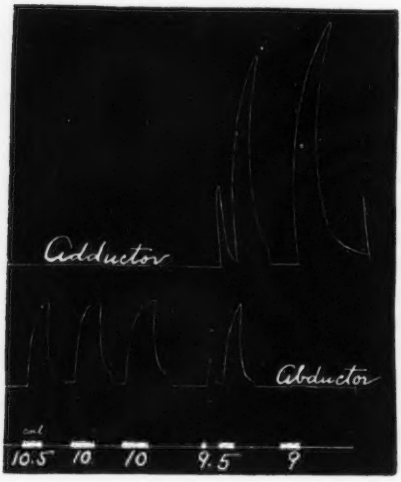

Fig. 1

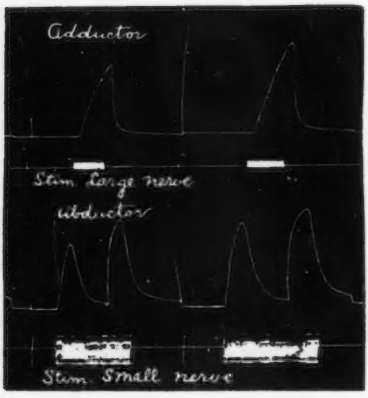

Fig. 2

Fig. 1. Record from blue crab showing usual order of events with increasing strength of stimulus. Levers were attached to the muscles.

Fig. 3. Repeated induction shocks applied to small and large nerve supplying the lobster's claw muscles. Contractions of the abductor and adductor muscles and reciprocal inhibition of the abductor are recorded.

A study of relative irritability by the chronaxie method, using condenser discharge, will be reported separately.

As stimuli of gradually increasing intensity, beginning with subliminal, are applied to the nerve supplying the claw muscles, in all forms examined, the order of events is similar to that previously described for the crayfish. Minimal stimuli cause contraction of the abductor and inhibition of the adductor. As a result the claw opens. Then a critical region is reached where simultaneous contraction of both muscles appears. The claw may open or close. With stronger stimuli the contraction of the abductor is

changed to inhibition. Biederman described a neutral zone of stimulus intensity where neither muscle contracts. In the forms investigated by us, at least under summer conditions, no true neutral zone was found. Figure 1 shows the usual order of events when the muscles are connected to separate levers. As Biederman found for the crayfish, it is more difficult to show inhibition in the adductor than in the abductor. This may be due either to the relative sensitivity or the relative numbers of the two kinds of fibers. To show definite inhibition, it is necessary that a contraction or residual tonus be present. In the forms studied by us, this tonus is slight and the amount of inhibitory relaxation is small. The inhibition is shown in figure 2, A and B. It is best demonstrated by producing a weak tetanus of the abductor and then suddenly superimposing a stronger stimulus. For the duration of the stronger stimulus the tetanus is inhibited (fig. 3).

It is difficult to explain certain features of the reversal of excitatory and inhibitory effects. It is easy to understand that given a lower threshold of excitability the abductor-excitatory and adductor-inhibitory fibers will be excited by a stimulus which is subliminal for other fibers. As the stimulus strength increases to the critical region, both sets of fibers are stimulated and the antagonistic muscles may both contract. Beyond this the causal relationship is not so clear. The adductor-excitatory and abductor-inhibitory fibers dominate when moderately strong stimuli are applied. Thus excitation is dominant in one muscle (adductor), and inhibition in the other (abductor). It is not clear just why this occurs. It may be that the dominant fibers are more numerous and quantitatively overshadow the others or some other mechanism may operate to render the opposing fibers less effective as stronger stimulation is employed. If when the strength of stimulation is increased the fibers having the lower threshold can be supposed to respond repetitively to each shock (Forbes and Gregg, 1915) then a condition analogous to "Wedensky inhibition" may possibly be established in these pathways. This could account for the observed facts.

It does not appear to have been previously recognized that the selective excitability and reciprocal inhibition is not limited to the claw muscles but is general in the appendages of arthropods. If Mangold's description of dual nerve supply to all striated muscles can be taken to indicate a similar functional control, it is probably general for all skeletal musculature in this group of invertebrates. At least as far as we have examined, the various joints which are moved by antagonistic flexor and extensor muscles may each be flexed or extended depending on the character of the stimulus. The extensors like the abductor or opener of the claw are stimulated by weak stimuli which inhibit the opposing flexors. With stronger stimuli the typical reversal takes place with strong flexion of the joints.

Final convincing proof that the reciprocal inhibition is activated through the dual nerve supply is found in the lobster. In this arthropod, a larger and a smaller nerve root arise from the ventral nerve cord and pass as separate nerves to the claw muscles. Although these nerves have been described (Herrick, 1911), we can find no mention of the employment of this fact in a physiological analysis of inhibition. Under the binocular dissecting microscope, it is possible to isolate the two nerves and to stimulate them separately. Under these conditions stimulation of the large nerve produced contraction of the adductor muscle with inhibition of the abductor and flexion of other joints. Stimulation of the small nerve caused contraction of the abductor, with opening of the claw, and extension of other joints. These results are not reversed by any change in the stimulus. We have here, then, definite poof that separate excitatory and inhibitory fibers supply each muscle. In the lobster, the two sets which supply each muscle run separately instead of being grouped together in a common nerve trunk. In only one instance out of a large series have we found a mixing of the fibers. In one instance weak stimulation of the large nerve caused contraction of both muscles. It is evident that the lobster's claw furnishes an exceedingly useful preparation since in this form only, of those examined, it is possible to stimulate separately the excitatory or inhibitory fibers to either of the claw muscles. Reciprocal inhibition is easily demonstrated by stimulating the large nerve while a tetanus is being maintained through the small nerve. The effect is shown in figure 3.

In a study of peripheral inhibition in the claw, the investigation of the effect of changes of frequency of repetitive stimulation obviously suggests itself. For this purpose, we used the "vacuum-tube stimulator." By its means, the rate of stimulation may be readily altered from about one in two seconds to several thousand per second. It was found that the two sets of fibers show the same selective response to changing frequency as to changing strength of stimulation. The abductor-excitatory and adductor-inhibitory fibers were more easily stimulated by the higher frequencies. Above a certain critical rate, the adductor-excitatory and abductor-inhibitory fibers were inexcitable even to strong currents while below this frequency they were more easily excited. At the critical frequency both sets of fibers were excited and the claw opened or closed depending on the strength of stimulus. At a lower rate only closing was found. The critical frequency varies with different species and with the condition of the preparation but generally ranged around 90 per second for the spider crab and 150 per second for the blue crab. In the lobster also the small nerve is more excitable to high frequencies. We have not been able to determine the range within which the response in muscle and nerve follows the stimulation frequency. In the upper ranges we may be well beyond the capacity of nerve and muscle to follow. This relationship is being studied further.

Some of our most interesting results were obtained with stimuli of such intensity or frequency as to fall in the "critical region." Superficially, at least, these results resemble, in a number of details, the features of reflex spinal conduction as described by Sherrington (1906) and later discussed by Forbes (1912) (1922). One of these points which characterize the arthropod response is the prominence of "summation." Noted first by Richet and later by Piotrowski, it was proved by Lucas (1917) to be a "summation of propagated disturbances" rather than a "local summation of inadequate stimuli." It is a much more conspicuous feature of the response of the crab's claw than of the nerve-muscle of the frog.

When stimuli near the minimal value are applied at a rate of about 5 per second, thirty or more separate effects may be summated in a step-like rise of the contraction curve. Summation is also an interesting feature of the response to stimuli which are near the "critical value" for intensity or frequency. At such stimulation values, where both sets of fibers are effective, the usual quick contraction may be replaced by a slow steady rise which seems like a true algebraic summing of effects. Sometimes this slow rise is in rhythmic steps which do not correspond to the rhythm of stimulation (fig. 2, C and D).

Forbes (1912) has called attention to the general tendency of antagonistic impulses arriving at reflex centers to produce rhythmic activity. His figures show oscillations varying from slight irregular tremors to somewhat irregular but well marked rhythms. These result from the simultaneous stimulation of excitatory and inhibitory afferent nerves. Records may be obtained from the muscles of the crab's claw which almost exactly duplicate Forbes' results. In many instances, we obtained rhythms much more regular than those figured by him. These rhythms do not correspond with, and seem to have no direct relation to the rhythm of stimulation. Rhythms of from 5 to 8 per second have been frequently observed (fig. 2, C and D).

The simultaneous excitaton of nerve fibers producing mutually antagonistic effects produces other "interference" phenomena. One of these is a marked "delay" or lengthening of latent period (fig. 2, E and I). This delay may be as long as 1.2 second. The normal latent period of the nerve-muscle preparation of the crab is, of course, much shorter. Summation of inadequate stimuli may be a factor in this delay, but the contraction when it starts proceeds with the usual speed of the twitch rather than the slow rise associated with the summation of stimuli which, taken singly, are subliminal.

Another of these "interference" phenomena is the sudden termination of contraction or "arrest" while the stimulus still continues (fig. 2, F). It is not unlike the cutting short of reflex central discharge by the excitation of an antagonistic reflex. The cutting short of contraction occurs

too early and relaxation is too abrupt to be accounted for on the basis of simple fatigue. In some way during the course of a tetanic contraction the inhibitory mechanism suddenly becomes effective just as in the first

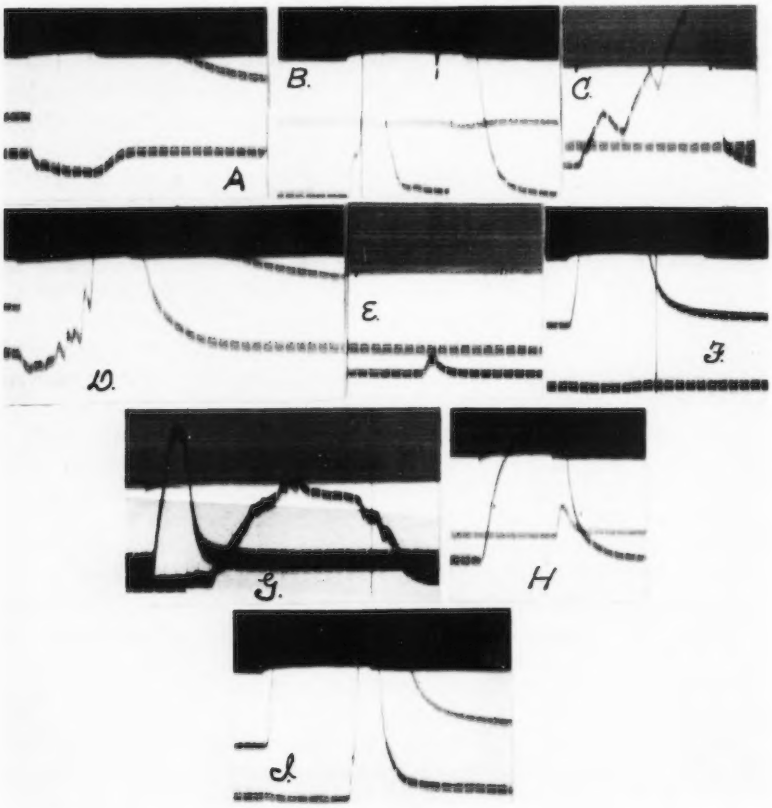

Fig. 2. Optical records. Time in $\frac{1}{5}$ second indicated by interruptions of the beam of light. Breaks in the upper line of shadow indicate time of commencement and cessation of stimulation. Upper lever line records movement of the adductor. Line at bottom indicates movement of the abductor.

A. Contraction of adductor and inhibition of abductor. B. Contraction of abductor and inhibition of adductor. C. Summation with rhythm of abductor. D. Initial inhibition of abductor followed by summation and rhythm. E. Delay. F. Arrest. G. Alternation or reversal. H. After contraction. I. Delay of abductor.

Note: Owing to the narrow paper, the upper limits of many contractions are not recorded.

case it delays the initiation of contraction. Further investigation is required to identify the factors determining these apparently opposite results. When the contraction of one muscle is "delayed" and the contraction of its antagonist is "arrested," a reversal or alternation in the activity of the two muscles appears (fig. 2, G). Levers attached to the antagonistic muscles show that one muscle contracts and then relaxes as its antagonist begins its delayed contraction. Depending on the stimulus, the intact claw may either first open and then close or may briefly close and then open. Occasionally, this reversal is repeated and the result is not unlike the alternation of action seen in such spinal phenomena as the "scratch reflex."

Sherrington (1908) has described two phenomena associated with central inhibition to which he applied the term "successive induction." One of these is "rebound" and to the other Forbes (1912), has applied the term "subsequent augmentation." Both of these types of "successive induction" may be seen in the responses of the crab's claw. "Rebound" is seen in the greater rise of the tetanus after a brief period of inhibition (fig. 3). As in spinal reflexes, the rebound is greatest after brief inhibition and becomes less as the inhibitory stimulus is prolonged. It is replaced by depression after prolonged inhibition. "Subsequent augmentation" on the other hand often appears after somewhat prolonged inhibition. In the response of the crab's muscle it is seen as an "after-contraction" which follows the cessation of the stimulus (fig. 2, H). Under appropriate conditions either some process associated with excitation but held in check outlasts inhibition and becomes effective when the stimulus ceases, or some substance accumulates and exerts its effect after restraining influences or substances disappear. With the relatively simple arrangement at hand, it is impossible to evoke the aid of long paths or multiple synapses to explain the phenomenon.

Discussion. Functionally the skeletal musculature of the arthropod lies between the visceral and skeletal muscle of vertebrates. It is striated and possesses the ability to respond to single induction shocks (brief chronaxie), and the speed of vertebrate skeletal muscle together with summating powers and double innervation of visceral muscle. It also possesses some ability to maintain tonus independent of central innervation. It may be, as Lucas (1917) concluded, that there are two kinds of contraction produced through different elements in the nerve and muscle. The relation of these elements to inhibition and inhibitory fibers requires further investigation. The arthropod claw is unusually favorable material for the study of the factors concerned in peripheral inhibition as conditions are the simplest possible and automaticity does not enter into the problem as it does in the case of cardiac and visceral control. From the experiments reported in this paper it is clear that such preparations are capable of

duplicating phenomena previously considered characteristic of spinal behavior. Further work is planned to determine the limiting factors concerned in the various conditions described.

SUMMARY

Attention is called to the arthropod as a form presenting unusual advantages for the study of peripheral inhibition.

Phenomena associated with the reciprocal inhibition of antagonistic muscles of the crab's claw are described.

The general prevalence of this type of control of the skeletal musculature of arthropods is noted.

Selective excitability of inhibitory and excitatory nerve fibers to different rates as well as to different strengths of stimulation, is demonstrated.

Attention is called to the advantages, for physiological analysis, of the separate course of excitatory and inhibitory fibers to the antagonistic muscles of the lobster's claw.

Peripherally produced summation, rhythm, delay, arrest, reversal and alternation, rebound and after-contraction, are described. Superficially, at least, these resemble similar phenomena associated with central conduction in vertebrates.

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CALCIUM IN THE COAGULATION OF BLOOD

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The literature on the problem of coagulation of blood is very extensive and has been thoroughly reviewed many times in various journals, therefore a repetition seems rather unnecessary. Reviews have been made by Hewson (1770), Wooldridge (1886) Morawitz (1903, 1905), Mellanby (1908), Howell (1916), Mills and Guest (1921), and Mathews (1926).

In reviewing the problem of the coagulation of blood, one is impressed by the large number of theories offered to explain the process, the complicated nomenclature given to the various contributing factors, and the different functions attributed to the components of the blood. It is obvious from a general survey of literature on coagulation, that the greatest diversity of opinion lies in the nature, as well as the manner of formation, of thrombin and prothrombin, and the rôle played by calcium in the coagulation process. This paper contains a report of the work done in this laboratory during the past four years in an attempt to make more clear the mechanism involved in the coagulation of blood as far as the action of thrombin, prothrombin and calcium is concerned.

METHOD OF CALCIUM DETERMINATION. A great deal of the experimental work in this paper had to do with calcium and its relationship to thrombin and prothrombin; consequently, a reliable method of calcium analysis was necessary.

Clark (1921) probably has the most convenient method of calcium determination for plasma or serum. His method consists of precipitating the calcium from serum or citrated plasma as Ca oxalate by adding to a sample of the serum or plasma, one-half volume of ammonium oxalate and allowing it to stand over night, then centrifuging, decanting off the supernatant fluid and washing the precipitate with distilled water, and again centrifuging. Finally, the precipitate is taken up in normal sulphuric acid, heated to 75°C., and the oxalic acid formed is titrated with 0.01 N $KMnO_4$. He showed that the serum or plasma must stand in contact with the oxalate for sixteen hours for all the calcium to be precipitated. Clark checked this method by taking equal parts of a given sample of serum. In one part, the calcium was determined by the above stated

method; in the other part, the sample was ashed, the ash dissolved in hydrochloric acid, neutralized, and the calcium precipitated by ammonium oxalate. From this point on, the procedure was the same as the other method. The determination on the two samples was the same if the serum or plasma was allowed to stand in contact with the oxalate for sixteen hours or longer. The delay in the precipitation of the calcium was attributed to the time necessary for the combined calcium to be broken down to the ionic form.

Since it was desirous to use this method on thrombin solutions as well as serum and plasma (both citrated and heparinized) a more extensive study of the method was made in order to determine its variation. The procedure followed in making this study may best be shown by citing in detail one of the six experiments.

A large dog was bled into three vessels:

(1) Blood allowed to clot and serum taken therefrom.

(2) Blood drawn into heparin (1 mgm. to 5 cc. blood) and centrifuged.

(3) Blood drawn into potassium citrate (final concentration 0.4 per cent) and centrifuged.

Calcium determination done on each of serum and plasma samples, using three different methods, each method being done in triplicate; 25 cc. samples used for each determination.

The procedures, referred to as A, B and C, were as follows:

A. Placed in 3 large centrifuge tubes (25 cc. in each); 12.5 cc. 3 per cent ammonium oxalate added to each tube and allowed to stand 16 hours. They were then centrifuged and supernatant fluid decanted off; 35 cc. cold distilled water added, stirred and again centrifuged and supernatant fluid decanted off. The precipitate was then taken up in normal sulphuric acid, heated to 75°C. and titrated separately against 0.01 N potassium permanganate. Results are all expressed in milligrams per 100 cc.

B. Placed in 3 large tubes (25 cc. each); one-half volume 3 per cent ammonium oxalate added to each tube and allowed to stand 16 hours and centrifuged, precipitated, washed with cold distilled water, again centrifuged and supernatant fluid decanted off. Precipitate was then removed to a porcelain crucible and ashed in an open flame to destroy any organic matter that could have been carried down with the calcium oxalate and possibly interfere with the titration. The ash was then taken up in N H_2SO_4. Results expressed in milligrams per 100 cc.

C. Sample divided into three 25 cc. portions which were ashed separately in porcelain crucibles in an open flame. The ash was then taken up in 15 cc. of 10 per cent HCl, brought to a pH of 4 with NaOH, using bromphenol blue as an indicator. At this pH magnesium oxalate is not precipitated. To each tube was then added one-half volume of 3 per cent ammonium oxalate and allowed to stand over night. They were then centrifuged, the supernatant liquid drawn off, 35 cc. cold distilled water decanted off, and the precipitate dissolved in normal sulphuric acid heated to 75°C. and titrated with $KMnO_4$.

The heparinized plasma and citrated plasma were divided into 75 cc. parts and the same procedure followed. The results of the analysis may be easier seen in tabulated form. Calcium values are expressed in milligrams per 100 cc. The contents of the three samples are given in each case.

If we take the greatest variation in the same sample in the same procedure, e.g., serum, procedure C, second and third 25 cc. samples, we find a variation of 0.1 mgm. in 13.20, or an error of 0.75 per cent. This may be regarded as the greatest probable error of the method and no greater variation was found in other experiments.

If the calcium values for the different procedures are examined, it is seen that the results are well within the limit of experimental error. It should also be noted from these results that the calcium content of the two plasmas and the serum is practically the same. Whichever procedure is followed, therefore, the results are comparable with those of other procedures provided the pH is kept at 4 and the oxalate is allowed to stand in contact with the calcium-containing fluid for sufficient time. Fibrin,

TABLE 1

	A	B	C
Serum	13.00	13.20	13.28
	13.28	13.20	13.20
	13.28	13.28	13.30
Average	13.18	13.22	13.26
Heparin plasma	13.26	13.18	13.24
	13.28	13.28	13.28
	13.26	13.26	13.28
Average	13.26	13.24	13.26
Citrated plasma	13.22	13.28	13.26
	13.28	13.26	13.26
	13.28	13.24	13.24
Average	13.26	13.26	13.25

as was shown by Mills and Guest (1921) and confirmed by one of us (Loucks 1926), normally contains calcium and therefore theoretically, serum should contain less calcium than plasma. However, the amount of calcium removed from plasma in this way from 100 cc. blood would fall within the limits of error laid down above.

This calcium method was also checked on different thrombin solutions and similar results were obtained which will be presented later in the paper.

Does any combined calcium of blood exist in union with lipoid? A review of the literature on the nature of calcium in the blood tells us very little except that there are apparently two existing forms of the metal, viz., the ionized form and the non-ionized. The exact amount of each form is a very much disputed question. No light can be thrown on the subject by making calcium determinations on serum and plasma by oxalate precipita-

tion because as soon as the ionized calcium is removed as calcium oxalate, the combined must break down to ionized form to maintain the equilibrium, for an equilibrium must exist between the two forms. The experiments of Cushny (1919) and others seem to indicate that the calcium is bound with protein in the blood, but there seemed a possibility that some of it might be bound to the lipoid of the blood. The following type of experiment was carried out to determine whether or not this was the case. Large amounts of blood (usually 500 or 600 cc.) were used which would eliminate many possible errors in the method of calcium determination.

A dog was bled into two vessels as follows:

1. 500 cc. drawn into a separatory funnel containing 1200 cc. ether.
2. 500 cc. drawn into a beaker and allowed to clot. The clot was then broken up and thrown into 1200 cc. ether in a separatory funnel.

These vessels were then allowed to stand (with occasional shaking) for 24 hours; the ether layer collected and evaporated to dryness, the residue taken up in anhydrous ether, agaid evaporated to dryness and a third time extracted with anhydrous ether. The third ether extract was evaporated to dryness, and residue placed in a porcelain crucible and ashed in an open flame. The ash was taken up in 10 per cent HCl and the solution brought to a pH of 4, the Ca precipitated and determined by the usual method.

The average results of two such experiments are given below.

Ca content of ether extract of 500 cc. of whole blood—3.3 mgm.

Ca content of ether extract of 500 cc. of clotted blood—3.9 mgm.

These results show about 0.6 mgm. of calcium per 100 cc. of whole blood to be bound to ether extractable material and slightly more in blood which has clotted. The significance of these results as they would influence coagulation is rather hard to interpret except that they would suggest a formation of some calcium lipoid compound being formed during the process of coagulation. At least it shows that some of the so-called bound calcium is probably bound with lipoid instead of protein.

Has the lipoid calcium compound anything to do with fibrin precipitation? From Howell's suggestion (1916) the coagulation process may be a colloidal precipitation of fibrinogen brought about by an oppositely charged colloid, and from the numerous observations that lipoid and calcium are essential to the coagulation process it seemed that the precipitating colloid might be a calcium soap. Practically the only soluble calcium soap known is the calcium soap of linolinic acid. Linolinic acid, however, is so readily oxidized that it is impossible to prepare the calcium soap from the pure acid before it is oxidized. Therefore, a potassium soap was made by saponifying linseed oil (raw) with KOH. The soap was neutralized and $CaCl_2$ added. The insoluble calcium soap was precipitated, but the soluble calcium soap remained in solution. This solution (after the insoluble substances had been filtered off) was used for these experiments.

Calcium ricinolate was prepared in the same manner from pure sodium ricinolate. The following is an experiment of the type used in testing the coagulating properties of these soaps. Blood was drawn from the femoral artery of a dog into heparin, then centrifuged and the plasma used in the experiment.

The tissue extract used was prepared from beef lung which had been perfused through the pulmonary artery with several liters of Ringer's fluid. The lung was ground and extracted for twelve hours with 0.9 per cent NaCl. This process would reduce the possibility of there being any thrombin left from the clotted blood in the vessels, but it is impossible to remove the lymph in a tissue. Since lymph clots, thrombin must be formed and consequently one must consider the possiblity of all tissue extracts containing thrombin.

TABLE 2

PLASMA	COAGULATION TIME
cc.	
3 + 0.3 cc. of 0.9% NaCl	Control, no clot in 6 hours
3 + 0.1 cc. of 1% $CaCl_2$	No clot
3 + 0.2 cc. of 1% $CaCl_2$	No clot
3 + 0.3 cc. of 1% $CaCl_2$	No clot
3 + 0.5 cc. of 1% $CaCl_2$	No clot
3 + 0.2 cc. tissue extract	No clot
3 + 0.4 cc. tissue extract	No clot
3 + 0.1 cc. $CaCl_2$ + 0.1 cc. tissue extract	Clot in 20 minutes
3 + 0.2 cc. $CaCl_2$ + 0.2 cc. tissue extract	Clot in 8 minutes
3 + 0.1 cc. Ca soap (linseed oil)	No clot
3 + 0.3 cc. Ca soap (linseed oil)	Clot in 1 hour
3 + 0.5 cc. Ca soap (linseed oil)	Clot in 1 hour
3 + 0.1 cc. Ca ricinolate	No clot
3 + 0.3 cc. Ca ricinolate	No clot
3 + 0.5 cc. Ca ricinolate	No clot

In a way, the above experiment is not conclusive because the clotting of heparin plasma was not always brought about by the Ca soap of linseed oil. In no case was a clot formed with the Ca ricinolate. A similar experiment was carried out, using a fibrinogen solution prepared from citrated plasma by precipitating the fibrinogen by half saturation with NaCl, dissolving in distilled H_2O and re-precipitating with NaCl with similar results.

The results with the fibrinogen solution were about the same as those of heparinized plasma, i.e., occasionally a clot could be formed with calcium soap. While it is possible that the precipitating agent in fibrin formation might be a calcium-lipoid compound formed from blood Ca and blood lipoids, our results show that all Ca soaps do not act as thrombin.

Substances needed to clot fibrinogen solutions and heparin plasma. Another observation made from the results of table 2 was the materials needed to coagulate heparinized plasma and fibrinogen solutions. Notice must be taken that neither calcium salts nor tissue extract (as prepared from perfused lung) will clot heparinized plasma and the same is true of fibrinogen solutions. However, if the tissue extract is prepared from lung as it comes from the abattoir without removing the blood from the vessels by perfusion, it will clot heparin plasma or fibrinogen solution. The coagulating agent in this case is probably the thrombin from clotted blood left in the vessels.

The tissue extract prepared from perfused lung will clot heparin plasma or fibrinogen only when calcium is present. The calcium chloride alone is ineffective in bringing about a precipitation of fibrin. The fact that it takes both tissue extract and calcium to precipitate fibrinogen from solution in the form of fibrin, indicates that the coagulant of fibrinogen must be a tissue calcium compound. Work which would substantiate this suggestion has been presented by one of the writers (Loucks, 1926). Below is cited one of seven experiments of this nature.

Tissue extract was prepared by grinding perfused beef lung with sand and extracting it with 0.9 per cent NaCl. A 1 per cent $CaCl_2$ solution was used as source of calcium and fresh oxalated dog's plasma as a coagulating substance.

1. 5 cc. plasma + 0.2 cc. $CaCl_2$—clot 11 minutes
2. 5 cc. plasma + 1 cc. tissue extract—no clot 9 hours.
3. 5 cc. plasma + 1 cc. tissue extract + 0.2 cc. $CaCl_2$—clot 4 minutes.

10 cc. of tissue extract were then treated with 2 cc. $CaCl_2$ and the following procedures employed:

1. 5 cc. plasma + 1 cc. of above mixture—clot 4 minutes, 35 seconds.

The remaining tissue extract-$CaCl_2$ mixture was then divided into two parts; to part (A) was added 1 cc. 10 per cent potassium oxalate, let stand for 2 hours, centrifuged and excess oxalate dialyzed out. Part (B) was dialyzed in a collodion membrane for 96 hours.

2. 5 cc. plasma + 1 cc. of above mixture (A)—no clot 11 hours.
3. 5 cc. plasma + 1.5 cc. of above mixture (A)—no clot 11 hours.
4. 5 cc. plasma + 2 cc. of above mixture (A)—no clot 11 hours.
5. 5 cc. plasma + 1 cc. of above mixture (B)—clot in 14 minutes.
6. 5 cc. plasma + 1.5 cc. of above mixture (B)—clot in 11 minutes.
7. 5 cc. plasma + 2 cc. of above mixture (B)—clot in 11 minutes.

It must be noted from these experiments that calcium can be removed from the tissue extract—$CaCl_2$ mixture by oxalate; but it is impossible to remove it by dialysis, which would indicate that the calcium was bound in some way to the tissue extract and can be withdrawn by the oxalate ion. In each case, when the tissue extract $CaCl_2$ mixture was dialyzed against distilled H_2O, an equal amount of $CaCl_2$ solution was placed in the same type of tube and used as a control; calcium disappeared from the

tube in about thirty-six hours. The above experiments would indicate that calcium was essential to the final formation of fibrin.

These experiments were repeated, using fibrinogen solutions to eliminate the factor of prothrombin. The results were similar to those just tabulated. They indicate that Ca plus tissue extract gives thrombin.

The action of fluorides in inhibiting coagulation. Another phase of the problem to be investigated regarding the rôle of calcium in clotting was the action of fluorides such as sodium fluoride, in inhibiting coagulation. Most textbooks state that while coagulation is inhibited by addition of fluorides to blood, re-calcification fails to induce clotting. This, together with the fact that the calcium was not precipitated (even though CaF is fairly insoluble) led some investigators to believe that something besides calcium was inactivated by the fluoride ion. Rettger (1909), on the other hand, states that fluoride blood will coagulate on the addition of calcium if the calcium is added a small amount at a time. Experiments were devised to learn whether or not Rettger's observations were correct. Three different kinds of blood were used, i.e., human, dog and fish blood (dog fish). The human and dog blood were drawn into sodium fluoride to a final concentration of 0.3 per cent which is sufficient to inactivate 143 mgm. of calcium from 100 cc. of blood. The dog fish required a final concentration of 0.6 per cent.

The following is typical of eleven experiments used in this series. Blood was drawn from the carotid artery of an etherized dog into NaF to a final concentration of 0.3 per cent or enough to inactivate 143 mgm. of Ca from 100 cc. of blood. The blood was immediately centrifuged and the plasma placed in Wassermann tubes, 1 cc. in each, and $CaCl_2$ added, according to the table.

1. 1 cc. NaF plasma + 0.2 cc. $CaCl_2$ (72.6 mgm. Ca per 100 cc. plasma) + 1 cc. 0.9% NaCl—No clot 24 hrs.
2. 1 cc. NaF plasma + 0.4 cc. $CaCl_2$ (145.2 mgm. Ca per 100 cc. plasma) + 8 cc. 0.9% NaCl—No clot 24 hrs.
3. 1 cc. NaF plasma +0.6 cc. $CaCl_2$ (217.8 mgm. Ca per 100 cc. plasma) + 0.6 cc. 0.9% NaCl—Clot 3 min.
4. 1 cc. NaF plasma + 0.8 cc. $CaCl_2$ (290.8 mgm. Ca per 100 cc. plasma) + 0.4 cc. 0.9% NaCl—Clot 22 min.
5. 1 cc. NaF plasma + 1.0 cc. $CaCl_2$ (363 mgm. Ca per 100 cc. plasma) + 0.2 cc. 0.9% NaCl—Clot 40 min.
6. 1 cc. NaF plasma + 1.2 cc. $CaCl_2$ (435.6 mgm. Ca per 100 cc. plasma) − 0 NaCl —No clot 24 hrs.
7. 1 cc. NaF plasma + 1.2 cc. 0.9% NaCl—No clot 24 hrs.

Blood (20 cc.) was drawn by vena puncture from the basilic vein of one of the writers, into NaF so that the final concentration of NaF was 0.3 per cent. The blood was immediately centrifuged and 1 cc. of the plasma placed each in a series of Wassermann tubes and treated as follows:

1. 1 cc. NaF plasma + 0.2 cc. $CaCl_2$ + 1 cc. 0.9% NaCl—No clot 24 hrs.
2. 1 cc. NaF plasma + 0.4 cc. $CaCl_2$ + 0.8 cc. 0.9% NaCl—Clot 35 min.
3. 1 cc. NaF plasma + 0.6 cc. $CaCl_2$ + 0.6 cc. 0.9% NaCl—Clot 8 min.
4. 1 cc. NaF plasma + 0.8 cc. $CaCl_2$ + 0.4 cc. 0.9% NaCl—Clot 30 min.
5. 1 cc. NaF plasma + 1.0 cc. $CaCl_2$ + 0.2 cc. 0.9% NaCl—No clot 24 hrs.
6. 1 cc. NaF plasma + 1.2 cc. $CaCl_2$—No clot 24 hrs.
7. 1 cc. NaF plasma + 1.2 cc. 0.9% NaCl—No clot 24 hrs.

This procedure was repeated four times with similar results.

Blood was drawn from the heart of the dog fish into sodium fluoride. It was found that 0.6 per cent sodium fluoride was required to prevent coagulation, and the blood must be immediately centrifuged. The plasma was used in the same manner as in the above experiments, with the following typical of results obtained in eight such experiments.

1. 1 cc. plasma + 0.2 cc. 1% $CaCl_2$ + 1.6 cc. 0.9% NaCl—No clot 24 hrs.
2. 1 cc. plasma + 0.4 cc. 1% $CaCl_2$ + 1.4 cc. 0.9% NaCl—No clot 24 hrs.
3. 1 cc. plasma + 0.6 cc. 1% $CaCl_2$ + 1.2 cc. 0.9% NaCl—Clot 30 min.
4. 1 cc. plasma + 0.8 cc. 1% $CaCl_2$ + 1.0 cc. 0.9% NaCl—Clot 25 min.
5. 1 cc. plasma + 1 cc. 1% $CaCl_2$ + 0.8 cc. 0.9% NaCl—Clot 10 min.
6. 1 cc. plasma + 1.2 cc. 1% $CaCl_2$ + 0.6 cc. 0.9% NaCl—Clot 45 min.
7. 1 cc. plasma + 1.4 cc. $CaCl_2$ + 0.4 cc. 0.9% NaCl—No clot 24 hrs.
8. 1 cc. plasma + 1.6 cc. $CaCl_2$ + 0.2 cc. 0.9% NaCl—No clot 24 hrs.
9. 1 cc. plasma + 1.8 cc. 0.9% NaCl—No clot 24 hrs.

The above experiments are an exact confirmation of Rettger's work. In addition, it must be noted that about two or three times the chemical equivalent amount of calcium must be added than would combine with the NaF present. The reason for this is difficult to explain. It seems, however, as though it must be a mass action effect. It must also be noted that too much calcium inhibits the process.

The nature of calcium in the blood. The work of Vines (1921) which tended to show the existence of combined calcium in the blood, was repeated. Vines used human blood in his experiments and therefore was limited to the use of very small quantities, generally less than a cubic centimeter. In repeating this work, large quantities of dog's blood were used for each experiment. The procedure followed was to use large etherized dogs, insert a paraffined cannula into the carotid artery, remove a sample of blood for calcium determination, and then bleed into volumetric flasks to which a given amount of oxalate has been added. A clean cannula was used for every flask. The chemical equivalent of potassium oxalate necessary to inactivate all the calcium from blood, i.e., approximately 10 mgm. per 100 cc., is not sufficient to prevent clotting. One cubic centimeter of a 2.075 per cent solution of potassium oxalate would be required to inactivate 5 mgm. of calcium, or the amount present in 100 cc. of blood (blood 52 per cent plasma and 10 mgm. calcium per

100 cc. plasma). The amount of blood drawn in each trial was 100 cc. and enought K oxalate present to inactivate the number of milligrams of calcium indicated in the table. Potassium oxalate solution 4.15 per cent was used throughout, since 166 grams (gram molecular weight of potassium oxalate) would be required to inactivate 40 grams (gram molecular weight of calcium), or 0.0415 gram to inactivate 0.010 gram. Below is given a typical experiment of this series. In the last column, in parenthesis, is given the number of milligrams of calcium that the added oxalate should combine with.

1. 100 cc. blood + 1.0 cc. 4.15% K oxalate + 3.2 cc. NaCl—clot 6 min. (chem. equivalent of 10 mgm. Ca)
2. 100 cc. blood + 1.4 cc. 4.15% K oxalate + 2.8 cc. NaCl—clot 6 min. (chem. equivalent of 14 mgm. Ca)
3. 100 cc. blood + 1.8 cc. 4.15% K oxalate + 2.4 cc. NaCl—clot 6 min. (chem. equivalent of 18 mgm. Ca)
4. 100 cc. blood + 2.2 cc. 4.15% K oxalate + 2.0 cc. NaCl—clot 7 min. (chem. equivalent of 22 mgm. Ca)
5. 100 cc. blood + 2.6 cc. 4.15% K oxalate + 1.6 cc. NaCl—clot 6 min. (chem. equivalent of 26 mgm. Ca)
6. 100 cc. blood + 3.0 cc. 4.15% K oxalate + 1.2 cc. NaCl—clot 12 min. (chem. equivalent of 30 mgm. Ca)
7. 100 cc. blood + 3.4 cc. 4.15% K oxalate + 0.8 cc. NaCl—clot 20 min. (chem. equivalent of 34 mgm. Ca)
8. 100 cc. blood + 3.8 cc. 4.15% K oxalate + 0.4 cc. NaCl—no clot (chem. equivalent of 38 mgm. Ca)
9. 100 cc. blood + 4.2 cc. 4.15% K oxalate—no clot (chem. equivalent of 42 mgm. Ca)

In the seven experiments performed, between three and four times as much oxalate had to be added to the blood to prevent coagulation as should be sufficient to precipitate the calcium present. This is in accordance with Vine's work. The explanation, however, is very difficult to conceive. It is possible that it has to do with the chemical activity of the combined calcium. However, the law of mass action may enter into this and it is possible that this amount of oxalate is necessary to bring about a precipitation in this system.

Place of action of calcium. The exact rôle which calcium plays in the coagulation of blood can undoubtedly be studied better from its connection with thrombin than any other way, because it was shown by one of the authors (Loucks, 1926) that calcium is essential to the activity of the thrombin.

The following experiment was devised to determine whether or not thrombin was inactivated by oxalate through removal of the calcium, or whether the oxalate ion itself interfered with the coagulation process in a manner similar to that of other neutral salts. The latter is the function

attributed to the oxalate in its interference with coagulation in experiments such as those carried out by Mellanby (1908–09) and McRae and Schnack (1913) in which they added oxalate to different coagulating systems, dialyzed out the oxalate and found the coagulating agents as active as ever.

Many times, during laboratory demonstrations on coagulation, it has been noticed by men in this department that occasionally defibrinated blood (which, of course, contains thrombin) would not coagulate oxalated blood. This was attributed to the salt action of the excess oxalate. The following experiments throw some light on the apparent nature of the oxalate action.

The blood used in the experiment given below was obtained from an anesthetized dog. A paraffined cannula was placed in the femoral artery and 50 cc. were drawn into a clean beaker and whipped to remove the fibrin and obtain defibrinated blood. Blood was then run into ammonium oxalate solution until a final concentration of 0.1 per cent oxalate was obtained (oxalate blood). Two cubic centimeters of the oxalated blood were placed in a series of Wassermann tubes and treated as follows:

1. Control—2 cc. oxalated blood + 0.4 cc. 0.9% NaCl—no clot
2. 2 cc. oxalated blood + 0.4 cc. 1% $CaCl_2$—clot 7 min.
3. 2 cc. oxalated blood + 1 cc. defibrinated blood—clot 4 min.
4. 2 cc. oxalated blood + (1 cc. defibrinated blood + 0.3 cc. oxalate)—clot 4 min.
5. 2 cc. oxalated blood + (1 cc. defibrinated blood + 0.3 cc. oxalate, mixed and immediately added to the oxalated blood)—clot 6 min.
6. 2 cc. oxalated blood + (1 cc. defibrinated blood + 0.3 cc. oxalate which had been allowed to stand together for 30 sec.)—clot 5 min.
7. 2 cc. oxalated blood + (1 cc. defibrinated blood + 0.3 cc. oxalate which had been allowed to stand for 1 min.)—clot 11 min.
8. 2 cc. oxalated blood + (1 cc. defibrinated blood + 3 cc. oxalate which had been allowed to stand for 2 min.)—slight clot 20 min.
9. 2 cc. oxalated blood + (1 cc. defibrinated blood + 0.3 cc. oxalate which had been allowed to stand for 3 min.)—no clot 24 hrs.
10. 2 cc. oxalated blood + (1 cc. defibrinated blood + 0.3 cc. oxalate which had been allowed to stand for 5 min.)—no clot 24 hrs.
11. 2 cc. oxalated blood + (1 cc. defibrinated blood)—clot 4 min.

In tube 4 the defibrinated blood was added to the oxalated blood and then the ammonium oxalate added immediately. In this case, the excess oxalate did not interfere with the action of thrombin in the defibrinated blood on the fibrinogen in the oxalated blood. For the rest of the tubes, the oxalate was mixed with the defibrinated blood and allowed to stand for the times indicated. Usually, 10 cc. of defibrinated blood were placed in a test-tube and 3 cc. of 1 per cent ammonium oxalate added, and 1 cc. of the mixture immediately placed in tube 5 which contained oxalated blood. This did not interfere with coagulation. To tube 6 were added 1 cc. of the defibrinated blood-oxalate mixture after it had stood for 30

seconds. There was no appreciable interference of oxalate with clotting. However, after the mixture had stood for one minute as in tube 7, the clotting time was longer and in no case was a firm clot observed. At the end of two minutes only, a few fibrin threads could be noted in the tube containing oxalated blood. After standing three minutes in contact with excess oxalate, the defibrinated blood would not produce a clot in oxalated blood. This was not due to the formation of metathrombin in the defibrinated blood because the unoxalated defibrinated blood was active in coagulating oxalate blood after standing from twenty minutes to one-half hour. The oxalate apparently inactivates thrombin by removing calcium from it. This type of experiment was repeated many times with similar results.

Further evidence of this reaction is given by the same type of experiment, using oxalated plasma and serum. The oxalated plasma was obtained by centrifuging oxalated ox blood obtained in the same manner as the preceding experiment. The serum was prepared by centrifuging defibrinated ox blood.

1. Control—2 cc. oxalate plasma + 1 cc. 0.9% NaCl—no clot
2. 2 cc. oxalate plasma + 0.4 cc. 1% $CaCl_2$—clot 9 min.
3. 2 cc. oxalate plasma + 1 cc. serum—clot 7 min.
4. 2 cc. oxalate plasma + 1 cc. serum + 0.3 cc. oxalate—clot 8 min.
5. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate mixed and immediately added to oxalate plasma)—clot 7 min.
6. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate which had been allowed to stand for 30 sec.)—clot 7 min.
7. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate which had been allowed to stand for 1 min.)—clot 8 min.
8. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate which had been allowed to stand for 2 min.)—weak clot 14 min.
9. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate which had been allowed to stand for 3 min.)—weak clot 16 min.
10. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate which had been allowed to stand for 5 min.)—no clot 24 hrs.
11. 2 cc. oxalate plasma + (1 cc. serum + 0.3 cc. oxalate which had been allowed to stand for 10 min.)—no clot 24 hrs.

This has been repeated a number of times using both ox and dog's blood.

The same type of experiment was used with thrombin solution and oxalated plasma. The thrombin used in this work was prepared from fresh dog's serum by precipitating it with twenty volumes of 95 per cent ethyl alcohol and allowing six to eight weeks for the precipitation to take place. It was then filtered and the precipitate dried, then taken up in distilled water and the protein removed by repeated shaking with chloroform and centrifuging. The supernatant fluid contained the thrombin.

1. Control—oxalate plasma + 0.5 cc. NaCl—no clot 24 hrs.
2. 2 cc. oxalate plasma + 0.4 cc. 1% $CaCl_2$—clot 5 min.

3. 2 cc. oxalate plasma + 0.5 cc. thrombin—clot 8 min.
4. 2 cc. oxalate plasma + 0.5 cc. thrombin + 0.3 cc. oxalate—clot 8 min.
5. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate mixed and immediately added to oxalated plasma)—clot 9 min.
6. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 30 sec.)—clot 9 min.
7. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 1 min.)—clot 8 min.
8. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 2 min.)—clot 9 min.
9. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 3 min.)—clot 13 min.
10. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 5 min.)—clot 20 min.
11. 2 cc. oxalate plasma + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 10 min.)—no clot.

Another experiment of the same type, using thrombin solution and fibrinogen solution, is tabulated below. The fibrinogen solution used was prepared by precipitating it from citrated plasma with one-half saturation of sodium chloride, dissolving the fibrinogen in distilled water and re-precipitating with one-half saturation of NaCl. It was again dissolved in distilled water and precipitated a third time with NaCl. The final precipitate was taken up in distilled water and used in the following experiment:

1. 2 cc. fibrinogen + 0.4 cc. $CaCl_2$—no clot.
2. 2 cc. fibrinogen + 0.5 cc. thrombin—clot 10 min.
3. 2 cc. fibrinogen + 0.5 cc. thrombin + 0.3 cc. oxalate—clot 11 min.
4. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate mixed and immediately added to fibrinogen)—clot 10 min.
5. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 30 sec.)—clot 13 min.
6. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 1 min.)—clot 11 min.
7. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 2 min.)—clot 11 min.
8. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 3 min.)—clot 17 min.
9. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate which had been alowed to stand for 5 min.)—clot $\frac{1}{2}$ hr. (weak).
10. 2 cc. fibrinogen + (0.5 cc. thrombin + 0.3 cc. oxalate which had been allowed to stand for 10 min.)—no clot 12 hrs.

From the above experiments it is evident that the oxalate interferes with the action of thrombin, but acts by removing the calcium and not by a salt effect on the protein, because the oxalate must stand with the thrombin for some time to bring about its inactivity. Thrombin solutions behave the same way as serum or defibrinated blood except that the time

required for its destruction by oxalate is found to be longer. This may be due to the greater concentration of thrombin in the pure solutions than that in serum or defibrinated blood. The calcium of the thrombin could not be dialyzed out of the thrombin-calcium combination. Thrombin was dialyzed against distilled water for ninety-six hours without impairing its activity.

The fact that oxalate added to a mixture of fibrinogen, or plasma and thrombin, as in tube 4, does not interfere with clotting, suggests that the thrombin has greater affinity for fibrinogen than the calcium in the thrombin has for the oxalate; otherwise, the calcium would be removed and the thrombin inactivated. However, it was found that if an excess of oxalate was used in these experiments, that in all cases it would inhibit clotting. For example, if 0.6 cc. of the oxalate solution was added to the tubes instead of 0.3 cc., no clot would be observed in any of the tubes. This was probably due to the more rapid removal of calcium from thrombin. A number of samples of thrombin were tested for calcium and all gave positive results.

Sources of thrombin. The fact that thrombin contains calcium suggested the possibility of preparing a thrombin solution from tissue other than blood. Beef lungs were therefore obtained from the abattoir and three different procedures followed.

1. The unwashed lung was ground in a meat grinder, then ground in a mortar and extracted with 0.9 per cent NaCl (2 cc. for each gram of lung) for 8 hours with continued shaking. The mixture was then filtered through gauze and the filtrate centrifuged. The supernatant fluid was placed in 10 volumes of 95 per cent ethyl alcohol and allowed to stand 4 weeks. The mixture was then filtered and the precipitate dried and taken up in distilled water. Then the solution was shaken with chloroform, centrifuged and the filtrate kept in the icebox. This extract was designated as thrombin E.

2. A lobe of beef lung was perfused with 6 liters of Ringer's fluid while intermittently expanding the lung with air. The perfusate was clear after about $3\frac{1}{2}$ liters had passed through. This was ground and extracted as in (1) and the 0.9 per cent NaCl extract divided into two parts. One part was placed in 10 volumes of 95 per cent ethyl alcohol and allowed to remain 4 weeks. Then an extract was prepared in the same way as (1) which was designated as thrombin F.

3. To the other part of (2) was added enough 10 per cent $CaCl_2$ solution to give 15 mgm. Ca per 100 cc. of the tissue extract. This mixture was shaken for two days in a shaking machine and then placed in 10 volumes of 95 per cent alcohol and treated the same as (1) and (2). The final product was called thrombin G.

The three thrombin solutions, E, F and G, were tried on fibrinogen solution and compared with a thrombin prepared from fresh serum by alcohol precipitation.

The following is a table of the time required to precipitate fibrin from a fibrinogen solution.

	Coagulation time
1. 2 cc. fibrinogen + 0.5 cc. serum thrombin	8 min.
2. 2 cc. fibrinogen + 0.5 cc. thrombin E	22 min. (weak clot)
3. 2 cc. fibrinogen + 0.5 cc. thrombin F	few fibrin threads found in 1 hr.
4. 2 cc. fibrinogen + 0.5 cc. thrombin G	firm clot 15 min.

Thrombin G is a very active coagulant although a bit slower than the thrombin prepared from serum. Both thrombin E and F are far inferior to G which was prepared from the calcified tissue extract. The fact that thrombin F, prepared from perfused lung, is less active than thrombin E, can probably be explained by the presence of thrombin in the blood left in the vessels of the lung. Calcium determinations on these thrombins showed a calcium content as follows:

25 cc. thrombin E—0.7 mgm.
25 cc. thrombin F—0.3 mgm.
25 cc. thrombin G—0.9 mgm.

Here it must be noticed that the coagulation activity followed the calcium content of the thrombin. Thrombin G was inactivated by oxalate in the same type of experiment used on thrombin solutions and serum.

This is fairly conclusive evidence that thrombin is some calcium containing compound.

Does fibrin contain calcium? However necessary the calcium may be to thrombin action, it does not take an active part in the reaction, nor is it a chemical component of fibrin, as is shown by the following experiments. If calcium were a chemical constituent of fibrin it should be present in different fibrin samples in constant amounts. Fibrin was prepared in various ways, as follows, and calcium determinations done on the fibrin produced.

Ox blood was obtained from the abattoir in potassium oxalate, centrifuged, and the plasma used in the experiment as a source of fibrinogen. The tissue extract used was that of ground beef lung.

1. 400 cc. plasma + 400 cc. 0.9% NaCl + 28 cc. 5% CaCl—clot 4 min.

Fibrin washed through four changes of 0.9 per cent NaCl solution for two hours each and then through three changes of distilled H_2O of two hours each and dried to constant weight.

Weight of washed fibrin—4.8514 grams.

Calcium content—0.04%.

2. 400 cc. plasma + 35 cc. tissue extract + 365 cc. 0.9% NaCl + 28 cc. $CaCl_2$—clot 20 sec.

Fibrin washed as before.

Weight of washed fibrin—5.1012 grams.

Calcium content—0.068%.
3. 400 cc. plasma + 400 cc. tissue extract + 28 cc. $CaCl_2$—clot 20 sec.
Fibrin washed as before.
Weight of washed fibrin 5.2111 grams.
Calcium content—0.126%.

The calcium was determined by ashing a given weight of the fibrin, taking up in N/10 HCl, precipitating the calcium as calcium oxalate, and titrating with $KMnO_4$ after N sulphuric acid at 70°C. had been added.

In experiments of this type, of which nine were done, calcium was always found present in the dried fibrin prepared in this way but in variable quantities. It is noted that the percentage of calcium ranged from 0.04 to 0.126 per cent in this experiment. This is a wider range than found in other experiments, but there was never a constant amount in different samples.

Then the question arose as to what was the cause of the variation in calcium percentages in different fibrins. To eliminate the possibility of the variation being in the technique of the analysis, two or three determinations were done on each sample. These checked within the limit of error of the method.

Calcium determinations were then made on fibrins obtained in the following manner:

1. Whole blood was whipped at the abattoir and the fibrin obtained was washed in tap water until free from cells. It was then rinsed in 0.9 per cent NaCl solution and washed in distilled water.
Calcium content—0.086%.
2. Fresh blood was obtained from the abattoir, oxalated and centrifuged. The plasma was clotted by addition of calcium and the clot ground and washed in 0.9 per cent NaCl through three changes and then through four changes of distilled water. Five different lots of fibrin were prepared after this manner.
Calcium content no. 1—0.102 per cent.
Calcium content no. 2—0.186
Calcium content no. 3—0.09
Calcium content no. 4—0.132
Calcium content no. 5—0.116

This experiment was repeated three times with similar results.

Another lot of fibrin was obtained by stirring whole ox blood and an attempt made to purify it by more thorough washing. It was washed in tap water for three days, shredded, and washed in distilled water through six changes of one to two hours each. Calcium determinations were made on this and none was found to be present. The only difference in procedure was that the fibrin was more thoroughly washed.

To determine whether the calcium actually could be washed away from the fibrin, the following type of experiment was carried out.

1. 400 cc. ox plasma + 400 cc. NaCl + 28 cc. 5% $CaCl_2$—clot 2 min. 40 sec.

Fibrin was divided into three portions: the first washed through four changes of NaCl and two of distilled water, each for one hour; the second through four changes of NaCl and six changes of distilled water, each for one hour; the third through six changes of NaCl and eight changes of distilled water, each for one hour. The portions were then dried in an oven of 100° to constant weight. Total weight of fibrin—4.379 grams.

Calcium determinations are as follows: No. 1—0.091 per cent; no. 2—0.040; no. 3—none.

2. 400 cc. ox plasma + 35 cc. tissue extract + 28 cc. 5% $CaCl_2$—clot 30 sec.

Fibrin divided into three parts and washed and dried as in experiment 1. Total weight of fibrin—4.6867 grams.

Calcium determinations are as follows: No. 1—0.11 per cent; no. 2—0.071; no. 3—trace.

3. 300 cc. ox plasma + 400 cc. tissue extract + 28 cc. 5% $CaCl_2$—clot 12 sec.

Fibrin divided into three parts and washed and dried as in experiments nos. 1 and 2. Total weight of fibrin—4.8367 grams.

Calcium determinations are as follows: No. 1—0.118 per cent; no. 2—0.06; no. 3—none.

This type of experiment was repeated five times and shows undoubtedly that calcium can be removed from fibrin by washing and probably is not a chemical constituent of the protein.

Returning to the question of the necessity of calcium in the second phase of coagulation and its presence in thrombin, an attempt was made to prepare thrombin from fibrin which was calcium-free. Fibrin was obtained from whipping ox blood and washing in NaCl and distilled water until the calcium content was negligible. Then it was extracted with 8 per cent NaCl by intermittent shaking for two days. The mixture was then filtered, precipitated with equal volumes of acetone, filtered again and dried. The precipitate was then taken up in 0.9 per cent NaCl. Preparations of this type failed to produce an active thrombin. Control preparations in which the procedure was identical except that the fibrin was not washed, were very active in coagulating plasma and fibrinogen solutions.

Source of prothrombin. Now the question arises: What must be the substance, prothrombin, which, with calcium, will give rise to thrombin? As shown above, it may come from the tissues as well as from the blood. Howell believes it to be a substance in solution in the circulating blood, while Wooldridge, Morawitz and others, believe it to come from the formed elements of the blood, viz., leucocytes and platelets. Burke and Tait (1926) have shown definitely that ruptured platelets are active factors in the formation of fibrin threads. However, there is no evidence to show that prothrombin may not come from any of the aforementioned sources. In normal coagulation, probably the most potent source is from the ruptured platelets. In thinking of this liberation of prothrombin from platelets, the question arose as to the causative mechanism of the rupturing

platelet. There are possibilities that it might be a type of autolysis of platelets or a dissolution of the membrane, or that the membrane would alter a rupture under a change of surface tension. The latter seemed more probable because of DuNouye's work, in which he described a marked fall in surface tension of blood when shed. A few experiments along these lines were carried out although they did not pertain directly to the nature of thrombin or the rôle of calcium in the coagulation of blood. The DuNouye torsion balance was used to determine the surface tension of blood. The blood was drawn from an anesthetized animal through a paraffined cannula directly into the watch-glass of the torsion balance. Generally, five or six readings could be taken before the blood clotted, that is, if no anticoagulant was used. Where anticoagulants were used, the blood was drawn from the carotid artery into a volumetric flask containing a known amount of the anticoagulant. This was shaken and immediately poured into the watch-glass of the balance, several readings taken for a normal and then recalcification begun, 1 per cent $CaCl_2$ being added, one drop at a time, and surface tension readings taken between additions. Below is given a table of a typical experiment, using dog's blood. The temperature was kept practically at body temperature.

ANTICOAGULANT	AVERAGE READING OF SURFACE TENSION IN DYNES PER SQ. CM.	RECALCIFICATION—EACH COLUMN IS THE READING AFTER ADDING ONE MORE DROP OF 1 PER CENT $CaCl_2$								
		1 drop	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops	9 drops
None	47									
None	47									
Citrate	51	52	54	55	55	50	47	46	46	clot
Oxalate (ammonium)	48	48	49	45	45	44	clot			
Heparin	47	48	46	46	46	46	47	46	no clot	
Defibrinated blood	42	41	42	42	41	41	41			

From the above table it is noted that blood drawn into anticoagulants, especially citrates, has a much higher surface tension than freshly drawn blood. Also, that when enough calcium is added back to citrated blood to produce clotting, the surface tension falls about ten per cent. This alteration is undoubtedly beyond the limit of experimental error of the balance. However, the inaccuracy of the instrument would prohibit a closer study of these changes. The platelets were not counted in these experiments, but from the work of Burke and Tait, we know that platelets rupture when blood coagulates. Furthermore, most of the solutions used in counting platelets depend upon sodium citrate for preserving the platelets. Evidently the lowered surface tension produced by adding calcium is the causative factor in the bursting of platelets. This being the case, it is further evidence in favor of platelets being a source of prothrombin.

But since thrombin can be prepared from tissue juice and calcium, the so-called prothrombin probably exists in any potential source of tissue juice, be it body tissues or platelets, or white blood cells.

SUMMARY AND DISCUSSION. Fibrin, as was shown by Mills and Guest and confirmed by one of the writers, normally contains calcium, and therefore serum should contain less calcium than plasma. However, the amount of calcium combined in this way from the fibrinogen in 100 cc. of plasma would fall within the limits of error laid down above.

The fact that the calcium content of fibrin is not a constant quantity, varying under similar conditions, from 0.04 to 0.126 per cent and in Mills and Guest's analysis from 0.12 to 0.18 per cent, and that the calcium can all be removed from the fibrin by washing, indicates that the calcium is not an essential part of the fibrin but belongs in all probability to the thrombin.

However, it was shown that thrombin contains calcium; also that when this calcium is removed, the thrombin is no longer an active coagulant of fibrinogen. Consequently, one can say that the formation of fibrin by thrombin is not a union of the two substances, but possibly a precipitation of one colloid by another; that is, the fibrinogen is altered from the sol to the gel form by thrombin.

No doubt both the phospholipin and the protein might be slightly different from different tissue, thereby producing slightly different thrombins with different activities, and therefore, there is no need of assuming two types of coagulation. If the calcium is removed, the thrombin is inactivated. The protein part of the thrombin has heretofore been considered as coming from the so-called prothrombin or thrombogen. The assumption of the existence of such a substance as prothrombin seems rather unnecessary. From the work in this paper, one can conclude that prothrombin, if there is such a thing, is tissue juice; that is, in the presence of calcium, thrombin can be prepared from extracts of body tissues. Platelets could be considered as a source of prothrombin from this standpoint. This suggestion is borne out by the work of Burke and Tait.

It seems unnecessary to assume that there are two types of coagulation as Mills and Matthews (1925) believe; that is, that blood can be clotted either by the normal mechanism of thrombin formation or by tissue fibrinogen and calcium. From the experiments in which thrombin was prepared from tissues, we see that it is very probable that there is only one thrombin. When blood is shed, the tissue juice from the wound or the content of ruptured platelets combines with the calcium of the blood to form thrombin. There is no evidence in the literature for assuming the existence of a separate chemical substance such as prothrombin other than that just stated. Prothrombin cannot be isolated in pure form as can thrombin as was shown by Rettger's work (1909).

The mechanism which normally comes into play in clotting shed blood is probably the formation of thrombin from the platelets, although the broken body tissues probably aid materially in the formation of thrombin. This statement is made in view of the fact that mammalian blood, collected free from contact with tissues, will clot, but does so slowly.

The mechanism involved in the rupture of platelets is probably the marked fall in surface tension of blood when shed, assuming that the tension at the platelet plasma interface follows the tension at the plasma air surface. Oxalates, citrates and fluorides "preserve" the platelets and are used as anticoagulants in methods of counting platelets. Such substances also maintain a high surface tension of shed blood. Although reference to the original work cannot be found, a statement is made in Stewart's "Textbook of Physiology" (1918) that "in all so-called decalcified plasmas, as ordinarily obtained, blood platelets are present and the platelets disintegrate under the influence of calcium salts." This is very good evidence in support of the conclusions made from the surface tension measurements of decalcified and recalcified plasmas and undoubtedly the disintegration of platelets is due to alterations of surface tension.

CONCLUSIONS

1. The calcium method of Clark was checked against ashing methods and the amount of calcium was found to be the same within experimental error, in serum, citrated plasma and heparinized plasma, if the oxalate was allowed to stand in contact with the plasma or serum for sufficient length of time.

2. It was found that the ether-extractable lipoids held some calcium in combination with them, but all calcium soaps do not act as thrombin.

3. Thrombin is probably a calcium—"tissue juice" compound which alters the sol fibrinogen to the gel fibrin.

4. Prothrombin, if such exists, is any potential source of tissue extract, i.e., broken tissues—platelets or white blood cells,—and not a separate chemical entity of the circulating blood.

5. Rettger's work regarding the recalcification of fluoride blood was confirmed. Fluoride plasma will clot upon recalcification if the calcium is not added in excess.

6. Vine's results showing that three or four times the amount of oxalate necessary to precipitate the normal amount of blood calcium must be added to blood to prevent its clotting, were confirmed.

7. Thrombin is inactivated by means of oxalate. This is due to the removal of calcium and is not merely a salt effect. It seems impossible to remove calcium from thrombin by dialysis.

8. Fibrin ordinarily contains calcium, but this calcium may be removed by washing. The calcium of fibrin probably belongs to the thrombin.

9. Lowering of surface tension of blood is probably the causative factor concerned in the rupturing of platelets in shed blood.

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THE INFLUENCE OF BICARBONATE AND PHOSPHATE BUFFERS AND OF CO_2 ON THE FUNCTIONAL ACTIVITY OF THE PERFUSED FROG HEART

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The functional activity of the cardiac mechanism as to rhythm, contractility and tone, the rate of depreciation with time, as well as the qualitative and quantitative reactions to various pharmacologic agents depends very largely on the character of the perfusion medium which is used as a standard. The multiplicity of "Ringer's" solutions which vary as to the H-ion concentration, ionic character, buffering agents and CO_2 content, used by various authors for perfusion purposes consequently result frequently in conclusions which are typical only for the particular perfusate utilized. This difficulty, encountered in attempting to adapt or reconcile results which were obtained under slightly different conditions, suggested the need for a comparison of the cardiac reactions during the perfusion of a relatively standard Ringer such as the Howell solution (consisting of 0.7 per cent NaCl, 0.025 per cent anhyd. $CaCl_2$, and 0.03 per cent KCl) with those which occur with a similar medium, with the H-ion concentration, the aerating medium, the calcium level, and the bicarbonate or phosphate buffer content as variables. In addition, it seemed worth while to determine whether data of a similar nature obtained with intestinal preparations (Sollmann et al., 1928–1929) are transferable directly to the perfused heart.

METHODS. Howell's Ringer solution supplemented with 0.1 per cent dextrose (an addition which was found to prolong the period of optimal function) was perfused at room temperature into the inferior vena cava of the intact frog heart by the method described by Sollmann and Barlow (1926). This method exposes the entire heart simultaneously to the action of drugs under more nearly natural conditions as to pressure, etc., in the different cavities, than are obtainable with the excised (Straub) heart. The observations included the rate, amplitude of contraction and qualitative changes in tone.

The usual procedure of an experiment was to perfuse the normal solu-

tion until a constant rate, tone and amplitude had been established. The perfusion of a solution identical in all but one respect i.e., with regard to either the pH, the calcium percentage, type of buffer, concentration of buffer or aerating medium, was then substituted during a 15 to 30 minute period for the control solution and followed by a recovery period with the normal perfusate. A sufficient number of frogs were studied with each type of solution to discount the natural variability. Several successive perfusions were made on each heart but the type of the solution tested was constant for a particular experiment.

All data of a similar nature were tabulated in two ways. The gross changes were noted by grouping the total number of perfusions made with all hearts, and in addition the effects of successive exposures of the heart with time were observed by grouping only the 1st, 2nd, or 3rd perfusions on each heart. These tabulations were subsequently reduced to medians which have been used for illustration.

The influence of various concentrations of bicarbonate on the cardiac mechanism at pH 7.2. The substitution of a Ringer's solution (the pH of which was corrected by means of HCl or NaOH to 7.2) containing $NaHCO_3$ in concentrations ranging from 0.008 per cent to 0.12 per cent, for a NaOH corrected Ringer of the same pH resulted, during a 15 to 30 minute perfusion, either in a slight depression of the rate, excursions, systolic and diastolic tone or practically no demonstrable change. Higher concentrations of bicarbonate were not tested because of the almost immediate development of opacity on preparation of the solution, due to changes in the state of the calcium ions.

With 0.008 per cent bicarbonate, the excursion, rate and tone changes were parallel, i.e., showed a 6 to 7 per cent decrease after 25 minutes' exposure of the heart. The reaction to 0.016 per cent was rather irregular, i.e., 30 per cent of the first perfusions showed a greater depression than is evident either from the mean change of all experiments or from subsequent experiments on the same heart. The median terminal change, however, was one of depression of the excursions (3 per cent) and a somewhat more pronounced depression (9 per cent) of the rate. This difference in the character or degree of the change produced on the excursions and rhythm was consistently observed with all concentrations of bicarbonate above 0.008 per cent tested. Certain concentrations of $NaHCO_3$, i.e., 0.032 and 0.04 per cent, actually increased the extent of the excursions (median of all experiments show maximal changes of plus 5 per cent) above that established during the perfusion of NaOH Ringer's, although a compensatory rate decrease occurred simultaneously. In general, however, only insignificant quantitative differences exist between the cardiac reactions to any concentration of bicarbonate tested, since the maximal changes noted varied from a 5 per cent increase in excursions to a 13 per cent

decrease in rate. The tone changes invariably paralleled the inotropic alteration.

At pH 8.0. The substitution of a Ringer solution buffered by means of various concentrations (0.008 to 0.12 per cent) of bicarbonate (pH corrected by means of NaOH or HCl) for a NaOH Ringer of the same pH, resulted in a terminal depression of the amplitude, rate and tone. This depression was somewhat more pronounced, especially with the higher concentration, than was observed at a pH of 7.2. The median degree of change was slight (—5 per cent) with 0.008 per cent $NaHCO_3$, but increased in proportion to the increase in the bicarbonate concentration, so that with 0.12 per cent the depression was approximately 20 per cent.

The inotrope in general was less depressed than the chronotrope. This difference in the degree of change of the two phases may equal 40 per cent, although the usual divergence was between 2 and 16 per cent. With concentrations of bicarbonate between 0.016 and 0.032 per cent the inotrope may be increased during a 2 to 10 minute period. This stimulation however is only apparent since the terminal picture invariably is one of depression and even during the period of inotropic increase, a corresponding compensatory decrease in the rate is evident and terminally, i.e., after a 15 to 30 minute perfusion, depression of the rate, amplitude and tone below that observed during NaOH Ringer perfusion is the rule. Increasing the alkalinity from a normal level (pH 7.2) to a pH of 8.0 apparently increases the susceptibility of the heart to the depressant effects of bicarbonate. These changes in the cardiac function in the presence of bicarbonate are in contrast to those noted under similar conditions with Magnus intestinal preparations. The bicarbonate action on the heart is referable to the bicarbonate ion and is not due to changes in the calcium, to cardiac fatigue or to excessive alkalinity since the substitution of a bicarbonate free perfusate of the same pH results in an improvement of cardiac function.

A comparison of the cardiac reactions to Ringer's solution buffered with various concentrations of Na_2HPO_4 and to a similar solution buffered with $NaHCO_3$. The cardiac changes due to decalcification in the presence of sodium phosphate have been described previously by Burridge. Further studies however were deemed necessary in the light of the complex intestinal changes noted by Sollmann et al. (1928). These authors observed that the immediate effect of the addition of small quantities of Na_2HPO_4 to Locke solution tended to improve the movements of the excised intestine, although not as much as does $NaHCO_3$. Concentrations of phosphate above 0.048 per cent were directly depressant. Sooner or later however and often in the course of the experiment, the phosphate solutions, the dilute as well as the concentrated, were observed to undergo chemical changes, manifested by a fall of pH and the development of turbidity or

precipitation. The observed alterations of intestinal function were ascribed to decalcification.

A comparison of the reactions of the perfused frog heart to Ringer's solution of identical ionic content and H-ion concentration but buffered with bicarbonate or phosphate respectively was made for the purpose of comparing the relative changes produced in cardiac and intestinal function by similar changes in environmental conditions, i.e., the effects and buffering efficiency of Na_2HPO_4 both as to concentration of the phosphate and the age of the solution. The cardiac changes in the presence of phosphate differ in certain important respects from those noted with isolated intestine under similar conditions.

The procedure of an experiment was to prepare a standard 0.02 per cent $NaHCO_3$ buffered Ringer and a phosphate Ringer containing from 0.008 to 0.096 per cent $Na_2HPO_4 \cdot 12H_2O$. The pH of the phosphate solution was determined colorimetrically and that of the bicarbonate solution, which served as the standard, was adjusted to the same level by means of NaOH or HCl. The bicarbonate solution was perfused through the heart until the cardiac functions were constant, after which the phosphate solution to be tested was substituted during a 20 to 30 minute period. This was followed by a recovery period during the perfusion of the normal solution.

New or fresh phosphate. The substitution of phosphate for bicarbonate Ringer's during a 20 minute perfusion period results, with phosphate concentrations between 0.008 and 0.024 per cent, in a slightly improved inotrope and a diminished chronotrope. Concentrations of phosphate higher than 0.024 per cent depress both the height of the excursions and the rhythm. The median, maximal inotropic improvement noted varied from 4 per cent with 0.008 per cent, to 12 per cent with 0.012 to 0.024 per cent phosphate. The alteration of the inotropic function with depressive concentrations varied from a negative but almost insignificant change with 0.048 per cent to complete loss of spontaneous contractility and rhythm with 0.096 per cent. The degree of change noted was proportional to the increase in the phosphate concentration.

The rhythm of the heart was only infrequently increased by any concentration of phosphate tested and the median change invariably indicated depression. With concentrations of Na_2HPO_4 between 0.008 per cent and 0.048 per cent, the differences were rather insignificant, i.e., ranging from a 5 to 10 per cent decrease. Perfusates containing concentrations of phosphates above 0.048 per cent produced a marked chronotropic depression which was complete within 2 to 5 minutes with 0.096 per cent (fig. 1).

Substitution of the standard bicarbonate perfusate for the phosphate was followed by a recovery of the original rhythm and excursions within 5 to 15 minutes, when the phosphate concentrations had not exceeded

0.024 per cent. Recovery following the perfusion of higher concentrations of phosphate was imperfect. In general the contractility was depressed or affected to a smaller degree than the rhythmicity. The tone changes, as usual, paralleled the inotropic changes. With stimulant concentrations of phosphate, however, there was a difference in the response of the different parts of the heart to phosphate, evidenced by an increase in the diastolic tendency of the auricles and sinus while both the diastolic filling and the systolic emptying of the ventricle were definitely improved.

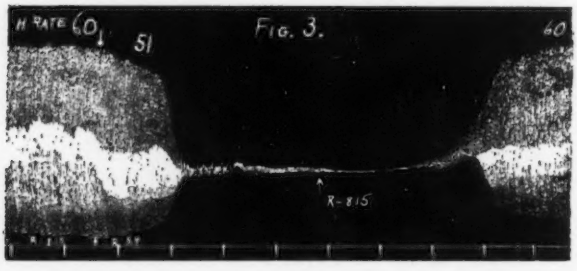
Old or aged phosphate. The cardiac changes produced by alternate perfusions of bicarbonate or phosphate buffered Ringer's containing concentrations of Na_2HPO_4 ranging from 0.008 to 0.096 per cent, originally of the same pH, were observed over a $2\frac{1}{2}$ hour period. The H-ion concentrations of the bicarbonate and phosphate perfusates were noted both at the beginning and end of each period.

The inotropic and chronotropic changes produced by 0.008 per cent Na_2HPO_4 were negligible with fresh solutions but resulted in a progressive depression as the perfusates aged, in spite of a small decrease in pH (from 7.8 to 7.5). With 0.012 and 0.024 per cent, cardiac function (excursions, tone and rhythm) was improved irrespective of the aging of the phosphate in a manner quite comparable to observed changes with Magnus intestinal preparations. The change however was due to improved environmental conditions (pH change) and not to decalcification.

The frog heart was not significantly altered by fresh 0.048 per cent phosphate concentrations but aged solutions and all concentrations above 0.048 per cent, resulted in a cardiac depression comparable to that observed under similar conditions with rabbit intestine. The heart changes observed were due in part to the increasing acidity of the phosphate perfusates and in part to decalcification. With aged, filtered (0.048 per cent) phos-

phate Ringer's, the depression was due primarily to the high acidity developed. With higher concentrations, i.e. >0.048 per cent, the primary change was due to decalcification, while a direct depressant action of the phosphate ion and the altered pH was of secondary, although significant importance.

The influence of the H-ion concentration on cardiac function. The importance of the gradual physical and chemical changes which take place in phosphate buffered Ringer's solutions with time necessitated, as an interpretative measure a study of the reactions of the perfused heart to alterations in the H-ion concentration. A standard 0.02 per cent bicarbonate Ringer (pH 8.0) was used as the control and stock solution. The pH of portions of this solution was then adjusted to various levels, i.e., from 7.8 to 5.4 by means of HCl. The procedure of an experiment was to prepare the heart and establish a constant functional activity during the perfusion of the control solution. The perfusates of greater H-ion concentration



were then substituted for the control solution for a unit length of time after which a recovery period was allowed by resubstituting the control perfusate, as illustrated in figure 2, in which the interrupted block represents the degree of recovery observed.

The gradual reduction of the alkalinity of the perfusate from 8.1 to 7.2 resulted in a slight but definite progressive improvement in cardiac function. This stimulant action, due to the establishment of more optimal environmental conditions was evident on reducing the pH from 8.1 to 7.8 or 7.5 and was reflected by a primary increase in rate (ranging from 4 to 12 per cent) and tone, and a variable alteration in excursions depending on the degree of the chronotropic change. The improvement noted on further reduction of the pH 7.2 was maximal and was reflected primarily as an increase in the excursions and tone, and an insignificant rate reduction.

Alteration of the pH of the perfusate from the alkaline to the acid side resulted in a progressive cardiac depression depending on the extent

of the pH change. The depression of the rate, tone and amplitude was small on perfusing a solution of pH 6.8 to 6.9. With a pH of 6.5 the several observations were diminished almost equally (16 to 30 per cent). The

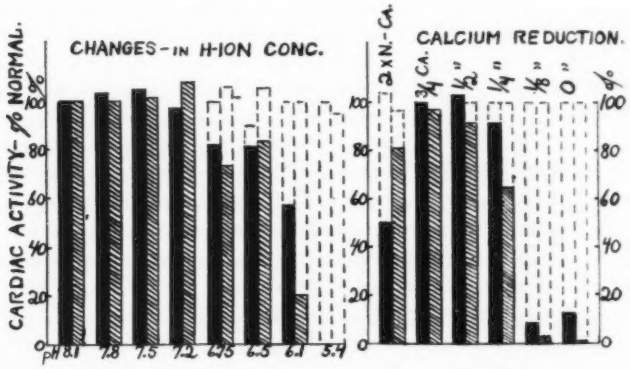


FIG. 2.

FIG. 4.

SOLID BLOCKS = RATE
HATCHED " = EXCURSIONS.
INTERRUPTED " = RECOVERY WITH NORMAL RINGER.

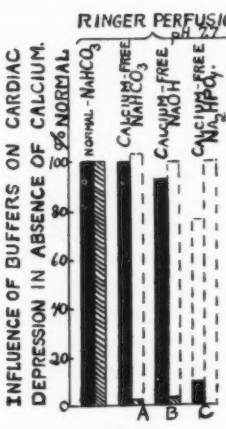


FIG. 5.

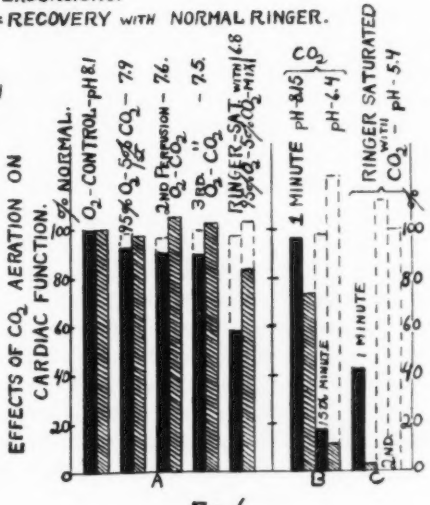

EFFECTS OF CO2 AERATION ON CARDIAC FUNCTION.
% NORMAL.
O2 - CONTROL - pH 8.1
95% O2 - 5% CO2 - 7.9
2ND PERFUSION - 7.6
O2 - CO2 - 7.5
3RD " - O2 - CO2
RINGER-SAT. WITH 95% O2 - 5% CO2 MIX 6.8
1 MINUTE pH 6.15
CO2
pH - 6.4
1½ MINUTE
2ND MINUTE
RINGER SATURATED WITH CO2 - pH 5.4
1 MINUTE
2ND
A B C

FIG. 6.

inotropic phase was decreased initially and to a marked degree in certain experiments, but in general to a somewhat greater degree than the rate. These differences after 30 minutes were insignificant. Reduction of the

pH from 8.1 to 6.1 or to 5.4 impaired primarily the contractility of the heart and the rate secondarily (fig. 3). The diminution in the height of excursions (85 per cent after 2 minutes at 6.1 and 96 per cent after 1 minute at a pH of 5.4) was accompanied by a maximal dilatation of the sinus and auricles. The rhythm on the other hand was decreased less markedly with the same range of change but the degree of depression tended to increase with time.

The influence of the calcium percentage of the perfusate on cardiac activity (fig. 4). The perfusion of a bicarbonate Ringer (pH 7.7) containing an excess of calcium (0.05 per cent or twice the normal concentration) produced a marked initial reduction of excursions during the first minute of exposure and subsequent recovery to 81 per cent normal by the fifth minute. The heart rate on the other hand was initially increased (12 per cent) during the first half minute of perfusion and rapidly reduced thereafter so that the values were 60 per cent normal at the second and 50 per cent normal after 5 minutes' exposure. Recovery on changing to a normal perfusate was complete. The chief terminal effects of calcium excess were marked rate reduction, decreased auricular tone, increased systolic tendency of the ventricle, and irregular block and a secondary decrease in contractility.

Reduction of the calcium content of the normal perfusate, on the other hand, resulted in a primary reduction of the excursions and a secondary reduction of the rhythm. The changes however were small over a rather wide range. Reduction to $\frac{3}{4}$ normal produced no significant change in either the ino- or chronotrope. Reduction to $\frac{1}{2}$ normal (0.0125 per cent) resulted in a small increase in the rate and a somewhat greater decrease in the excursions. These changes became maximal by the 5th minute and the degree of depression remained relatively constant during an additional 25 minute exposure of the heart to the low calcium perfusate. The perfusion of a Ringer solution containing $\frac{1}{4}$ the normal calcium percentage (0.00625 per cent) resulted in an initial small increase in rate which was quickly followed by a 10 per cent depression. The inotropic reduction was irregular but approximated 35 per cent by the 30th minute. Further reduction of the calcium level markedly reduced the excursions, tone and rate. The excursions and tone were reduced from 80 to 97 per cent during the first 5 minutes and progressively diminished thereafter. The rate was initially reduced relatively less than the excursions but after 20 to 30 minutes, the differences in the two observations were insignificant.

The influence of the character of the buffering agent on the cardiac reaction to calcium-free Ringer's solution. The influence of the perfusion of the frog heart with a calcium free solution, the pH of which was corrected by means of either Na_2HPO_4, $NaHCO_3$ or NaOH is illustrated by figure 5 A, B and C. The excursion changes differ negligibly, although the greatest depres-

sion was observed with Na_2HPO_4. The functional depreciation with time or depression of both excursions and rate, which develops in the presence of solutions containing the different types of buffering agents was greatest with phosphate, and least with bicarbonate. The differences in the cardiac reactions to the NaOH and $NaHCO_3$ Ringer's although slight appear sufficiently definite to indicate that the presence of the bicarbonate ion in low concentrations maintains a more optimal rhythm than NaOH. The rhythm and contractility of a heart completely depressed by phosphate (calcium free) Ringer's may likewise be definitely improved by substituting a bicarbonate calcium free perfusate.

The markedly greater depression produced by the di-sodium phosphate perfusate, C, than was observed in either A or B (fig. 5) indicates that the phosphate ion has a direct toxic action on the cardiac mechanism. This action is independent of changes in either the calcium content or state of the solution, or in the H-ion concentration.

Effect of CO_2 on the perfused heart. The influence of aeration of the perfusate with a mixture of 95 per cent O_2 and 5 per cent CO_2 is illustrated in figure 6 A. A Ringer solution buffered with $NaHCO_3$ (0.02 per cent) was used as the standard. Both Mariotte bottles were filled with this solution; one was connected with a spirometer containing the gas mixture while the other was aerated either with room air or with pure O_2.

Aeration of the perfusate with an O_2-CO_2 mixture resulted during the initial 5 minutes of perfusion in a 9 per cent increase in rate, but after 20 minutes a small ino- and chronotropic depression was the rule. During the second perfusion an increase in the systolic tendency resulted in an improved amplitude of contraction. The rate, on the other hand, compensated and was progressively depressed. With all perfusions subsequent to the first, the primary picture was one of depression in which the chronotropic changes were predominant. With a solution saturated with the gas mixture, the pH was altered from 8.1 to 6.8. Such a perfusate although depressing the rate to a much greater degree than the excursions, produced changes which were primarily due to the alteration of the H-ion concentration and are not attributable specifically to the CO_2 content of the perfusate.

The cardiac changes produced by perfusing a bicarbonate Ringer (pH 8.15) aerated with pure CO_2 are illustrated in 6 B and C. The depressive changes were marked and obviously were due to an alteration of the pH. Recovery of function on substituting a CO_2 free Ringer of standard pH, i.e., the same as that used at the beginning of the experiment, subsequent to the perfusion of the solution aerated with a mixture of O_2 and CO_2 or with pure CO_2, was rapid and complete and was followed generally by an improved activity. The functions invariably recovered both as to time and degree, inversely to the depression produced through alteration of the aerating medium.

SUMMARY AND CONCLUSIONS

The substitution of a bicarbonate buffered solution for a NaOH Ringer of a similar ionic content and pH resulted in a 6 to 7 per cent depression of the rate, tone and excursions of the perfused heart. The rate was decreased to a slightly greater extent than the excursions although individual experiments showed occasional irregularities. The degree of cardiac depression produced by concentrations of $NaHCO_3$ ranging from 0.008 to 0.12 per cent (pH 7.2) show only slight quantitative differences. The median changes with 0.008 to 0.024 per cent although showing depression were as a rule insignificant. These changes observed with the perfused heart show a marked divergence from the reactions of the isolated intestine under similar conditions. The intestinal rhythm has been shown to increase in proportion to the bicarbonate concentration, although the most optimal concentration appears to lie between 0.008 and 0.016 per cent, while the excursions in general are decreased reciprocally to the rate increase, (Sollmann et al., 1928; Rona and Neukirch, 1912). The cardiac depression produced by the range of bicarbonate concentrations studied was definitely greater at a pH of 8.0 than at 7.2, and with the more alkaline medium showed a qualitative relationship to the increasing concentration of $NaHCO_3$.

The substitution of a fresh phosphate for a bicarbonate buffered Ringer's of the same pH resulted, with phosphate concentrations between 0.008 and 0.024 per cent in a mean cardiac improvement. This improvement is quite similar to that observed by Sollmann et al. (1928), for the intestine. Phosphate concentrations above 0.048 per cent (the pH remaining constant) were invariably depressive and the negative cardiac change produced was proportional to the concentration.

The use of phosphate buffers in preparing perfusion solutions for the frog heart, although producing optimal reactions within a rather narrow range of concentrations, was found to be much less satisfactory as a stabilizing agent than bicarbonate since solutions containing the former buffer are subject to progressive changes in pH (an alteration which primarily explains the stimulant action observed with low concentrations), and in the state of the calcium. These changes become evident within 10 to 15 minutes, are progressive and are proportional in both time and degree of reaction to the phosphate concentration present.

A comparison of the correctives tested shows that although NaOH Ringer's produces a more optimal cardiac function than bicarbonate and the degree of functional depreciation with time is not extensive over a 4 to 6 hour period with either, the pH changes of the NaOH corrected solution even with pure O_2 as the aerating medium make such a perfusate unsuitable for quantitative studies over a period longer than 1 hour.

These changes are not encountered with $NaHCO_3$. The least efficient or suitable buffer tested was the phosphate.

Reduction of the pH of the perfusion solution from 8.1 to 7.2 resulted in a gradual improvement of cardiac function which was optimal at the lower pH. This improvement was reflected by a primary increase in rate and tone and a variable alteration in excursions which was a reciprocal of the chronotropic change. Further reduction of the pH progressively diminished the cardiac efficiency, i.e., the inotropic phase was decreased primarily and the rhythm secondarily.

The primary reaction of the heart to a perfusate containing 0.05 per cent $CaCl_2$ (i.e., double the standard concentration) was a marked chronotropic decrease, diminished auricular tone, a systolic tendency of the ventricle, an irregular block and a secondary inotropic depression. Reduction of the calcium content of the perfusate to $\frac{3}{4}$ of the normal percentage did not affect the cardiac efficiency significantly. Reduction to $\frac{1}{2}$ (0.0125 per cent) normal produced a small but significant depression. Further reduction markedly diminished the excursions and tone primarily and the rate secondarily. These differences, the extent of which were directly proportional to the degree of the calcium reduction, largely disappeared after prolonged exposure of the heart to the low calcium perfusate.

The cardiac reactions to perfusion solutions either saturated or aerated with carbon dioxide are directly attributable to the alterations of H-ion concentration which are produced.

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THE ULTRA-VIOLET TRANSPARENCY OF THE VARIOUS LAYERS OF HUMAN SKIN

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From former measurements on the penetration of ultra-violet light through human skin (1) it seemed evident that various layers of skin showed differences of transmission. These results were far from conclusive, however, because little errors in the determination of thickness, and small changes occurring between the preparation of the layers and the final experiment would result in comparatively great inaccuracies in the obtained results. Therefore, a simple new method was used which overcame the greater part of these difficulties and which exhibited the expected transparency differences in a very conspicuous way.

Frozen skin sections were obtained from the foot sole of fresh white and colored bodies, the thickness ranging from 10 to 70μ. These were placed between a quartz carrier and a quartz cover of $\frac{1}{5}$ mm. thickness. These sections were put on the slit of the quartz spectrograph with the surface and each layer of the skin put in a rectangular position to the slit.

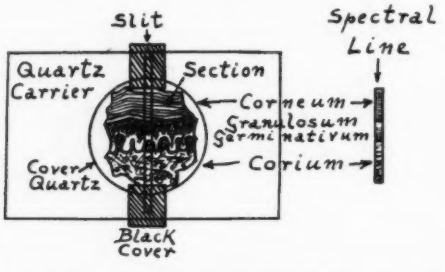

Fig. 1. Diagram explaining method of observation

The spectrograph was adjusted for exact stigmatic pictures. The light of the Kromayer lamp was sent through the skin and a series of spectrograms was taken with exposures ranging from 1 to 1000 seconds, and comparison spectra put on the same photographic plate with $\frac{1}{25}$, $\frac{1}{5}$ and 1 second exposures. The obtained photographs gave the following items:

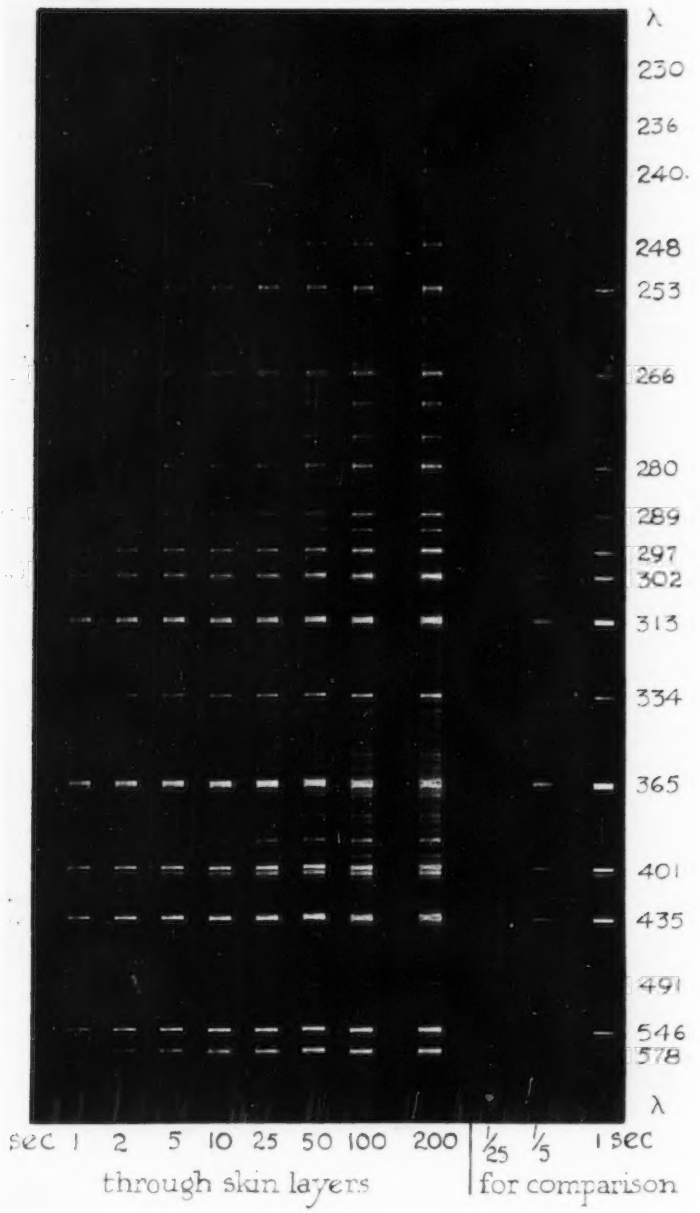

Fig. 2. Photographic plate showing comparison and transmission spectra of various skin layers obtained by varying exposure to Kromayer light.

1. Each spectral line showed the transmission of the various skin layers, the stratum corneum, (sometimes the lucidum,) the granulosum, germinativum, corium, all for one uniform thickness of these layers.

2. Each spectrum showed these results for all the Hg wave lengths between 578 and 230$\mu\mu$.

3. For every wave length and skin layer so many shades of optical density were present due to the different exposure time for each spectrum that transmissions between approximately 100 per cent and 0.1 per cent could be measured.

4. Each photographic plate gave the same results for another thickness, and for the same or other skin samples. The transmission was determined by comparison of the optical densities secured through the respective skin layer with those of the comparison spectra, with proper consideration of the Schwartzschild factor. The absorption coefficients were computed according to the formula $I_d = I_o\, 10^{-\mu d}$, with d in millimeters. The direct results of the measurements are compiled in table 1.

In the following this table is studied from various angles:

1. For all the photographic plates the average absorption coefficient of all the observed skin layers was calculated for the 4 wave lengths; 546–578; 313; 280; 248$\mu\mu$. The figures are given in the last columns of each division in table 1. In figure 3 these average absorption coefficients

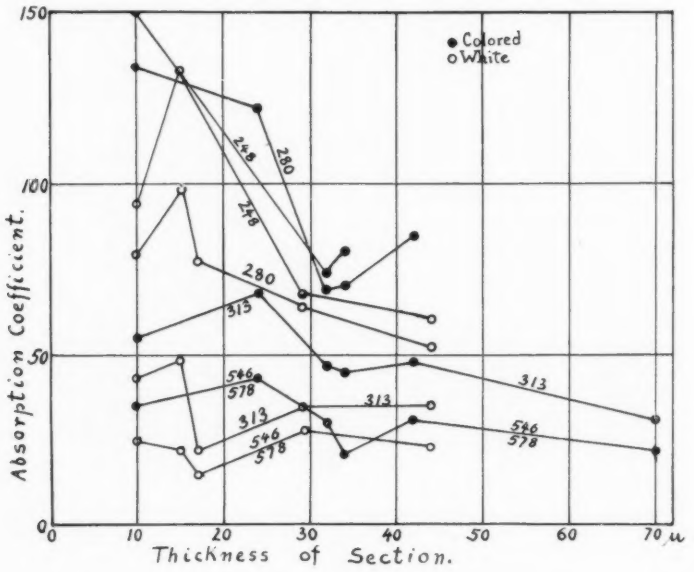

Fig. 3. Variation of absorption coefficient with thickness of sections

TABLE 1

λ	XXV 10μ wh.					XXII 10μ col.					XVII 15μ wh.				II 17μ wh.			VII 24μ col.					VI 29μ wh.				
	Corneum	Granulos.	Germinat.	Corium	Average	Corneum Upper	Corneum Lower	Granulos.	Corium	Average	Corneum	Granulos.	Corium	Average	Corneum	Corium	Average	Corneum Upper	Corneum Lower	Gran. + Germ.	Corium	Average	Corneum Upper	Corneum Lower	Gran. + Germ.	Corium	Average
546; 578	22	30	19	30	25	30	30	52	30	35	16	32	19	22	20	9	15	38	40	51	43	43	24	34	32	18	27
435; 491	16	30	16	40		25	30	60	30		16	47	28		30	12		46	50	52	46		24	34	32	18	
401; 405	19	32	16	52		25	30	60	30		16	65	35		20	13		51	67	67	51		24	34	36	24	
391	19	45	16	52		30	37	70	37		16	68	39		31	13		54	71	71	64		24	34	36	27	
365	22	52	16	52		34	37	70	37		16	72	44		24	9		54	71	71	64		27	35	34	27	
334	19	60	16	60		37	48	70	37		16	80	39		31	9		64	72	72	64		27	40	34	28	
313	22	60	26	63	43	43	52	82	46	55	19	80	47	49	31	13	22	67	72	72	67	70	31	40	36	32	35
302	30	77	34	67		66	70	86	68		27	83	47		31	13		73	78	78	67		34	49	38	34	
297	30	82	39	70		77	80	92	70		35	87	55		41	13		76	83	79	71		34	59	38	34	
293	39	82	52	73		77	96	104	70		46	87	63		60	18		93	106	100	71		37	65	53	38	
289	52	82	70	74		80	100	115	72		70	100	74		76	18		100	125	105	74		45	73	55	40	
285	60	90	74	74		100	155	170	78		92	120	84		95	24		115	140	130	76		55	90	57	43	46
280	61	105	76	76	78	115	155	170	76	134	99	115	84	99	130	24	77	125	140	135	76	122	59	94	59	45	
275	82	100	82	82		104	130	150	82		92	118	84		118	24		125	140	140	76		59	74	59	45	
265	77	100	80	89		100	130	170	80		72	120	81		118	24		115	130		80		58	58	53	53	
253	70	110	77	96		105	130	180	90		80	116	80		90	31		110	125		88		52	57	53	53	
248	73	122	80	100	94	122	155	222	100	152	92	156	92	135			—	120			93	—	59	65	73	70	67
245	77	152	89	109		160	190	240	110		112	156	100								112		70				
240	146	225	170	129		210	222	250	129		130	173	112								112		80				
238	190	250	178	140		222	240	280	134		152	200	135														
236				152					210				143														
234				164					250																		
230				182					280																		

λ	V 32μ col.							III 34μ col.							XII 41μ col.						XI 44μ wh.						IV 70μ col.				
	Corneum Upper	Corneum Lower	Lucidum	Gran. + Germ.	Germinat.	Corium	Average	Corneum Upper	Corneum Lower	Lucidum	Gran. + Germ.	Germinat.	Corium	Average	Corneum Upper	Corneum Lower	Lucidum	Granulos.	Corium	Average	Corneum	Lucidum	Gran. + Germ.	Corium Upper	Corium Lower	Average	Corneum	Granulos.	Germinat.	Corium	Average
546; 578	26	40	22	32	16	32	30	19	26	16	26	16	22	21	37	37	18	37	30	31	29	20	30	21	12	23	18	25	22	21	22
435; 491	29	40	25	31	19	32		30	41	32	40	21	25		37	37	20	56	30		29	23	30	24	14		18	25	22	21	
401; 405	32	44	29	32	19	32		31	44	32	40	23	27		37	37	20	56	34		33	26	34	26	16		21	27	27	26	
391	32	44	31	35	19	33		32	45	34	36	23	23		39	39	8	56	37		28	29	34	29	16		24	28	27	27	
365	38	47	32	38	22	38		34	45	34	36	26	33		42	42	18	56	37		36	33	39	29	22		25	30	28	28	
334	44	58	40	47	24	47		38	54		41	30	36		43	43	37	60	40		38	34	39	34	23		25	31	30	29	
313	53	66	40	50	24	50	47	45	59		47	36	41	45	45	49	40	62	44	48	39	36	40	36	24	35	29	32	31	29	30
302	55	70	45	50	26	50		45	62		50	41	44		45	50	40	54	45		42	38	42	38	30		30	37	34	29	
297	63	79	47	50	26	50		50	63		56	44	46		46	56	42	60	44		45	42	43	42	32		31	43	39	30	
293	70	90	50	55	28	50		60	70		56	44	50		47	65	45	73	49		50	42	45	45	34		36	50	42	31	
289	72	94	50	55	31	50		62	74		60	45	59		49	73			49		54	45	45	45	34		39	60	50	32	
285	76	100	53	60	35	52		65	80		60	50	63		55	80			55		65	47	47	47	36					33	
280	79	120	53	63	40	54	69	68	100		60	53	68	70	62	(90)		(100)	58	(80)	70	50	50	50	38	52	(60)	(70)		33	(54
275	77	120	60	63	44	60		70	100		65	60	68		66				60		75	50	51	52	40					35	
265	72	100	64	66	44	63		64	100		63	58	63		56				58		70	52	52	52	41					37	
253	63	94	63	68	48	66		64	110		63	59	63		56				58		70	54	67	67	41					37	
248	72	100	66	85	48	68	73	74	120		80	68	68	80	62				58		80	57	61	61	45	60					
245			75		50																				50						
240			85		53																				54						

are plotted against the thickness of the respective sections. Curves are plotted separately for white and colored specimens, although there was no pronounced difference of absorption in the sole of the foot. Most of these curves indicate an increase of the absorption coefficient from thick to thin sections. This fact shows that the exponential absorption law does not hold true exactly. The same observation was made in a previous study with J. Kunz (1).

We have offered three explanations for this occurrence, reflection, scattering and fluorescence. A pronounced reflection decreases the transmitted intensity independently of the thickness; hence it has a relatively greater effect upon thin sections. The amount of scattering depends upon the volume of irradiated tissue, and more scattered energy is preserved in a thicker layer. The fluorescence factor is practically eliminated in the photographic work with a discontinuous spectrum, as the fluorescent light does not coincide with the spectral lines of the mercury vapor. This observation makes the usual methods of calculating the distribution of energy from the absorption coefficient illusory and indicates that actual measurements with a specially designed method are required to settle the problem of light distribution inside of the tissue.

2. The absorption curves have been plotted for one characteristic plate, separately for the various layers of skin (fig. 4). These curves are entirely comparable because the sections have identical thickness.

The horny layer is very transparent in the visible and near ultra violet. At 300$\mu\mu$ a strong absorption band starts with a sharp maximum at 280$\mu\mu$. A pronounced absorption minimum is reached at 260$\mu\mu$, after which the absorption increases very rapidly to the extreme ultra violet. There exists a difference between the upper and the lower parts of the horny layer, as the absorption gradually increases with the depth. The extreme upper part of the horny layer shows marked irregularities with very strong absorption prevailing at those parts which are splitting off due to mechanical wear.

The stratum granulosum has an absorption curve, very similar to the one of the stratum corneum, but with greater absorption coefficients, particularly in the visible; farther toward the ultra violet the difference gradually disappears; the absorption maximum at 280$\mu\mu$ is evident, but not so pronounced.

The stratum germinativum is the most transparent, with the lowest absorption coefficients; the 280$\mu\mu$ maximum is just indicated.

The corium shows an absorption curve rather different from the others. In the visible the absorption is comparatively large, usually exceeding every other absorption except the one of the granulosum. The increase at 300$\mu\mu$ is rather slight; there is no absorption maximum at 280$\mu\mu$; in

the far ultra violet its absorption is smaller than the one of the other layers except the germinativum. In every case the corium curve crosses the curve of the horny layer near 300$\mu\mu$. The corium absorption is the least homogeneous of all layers. The figures given above are the average

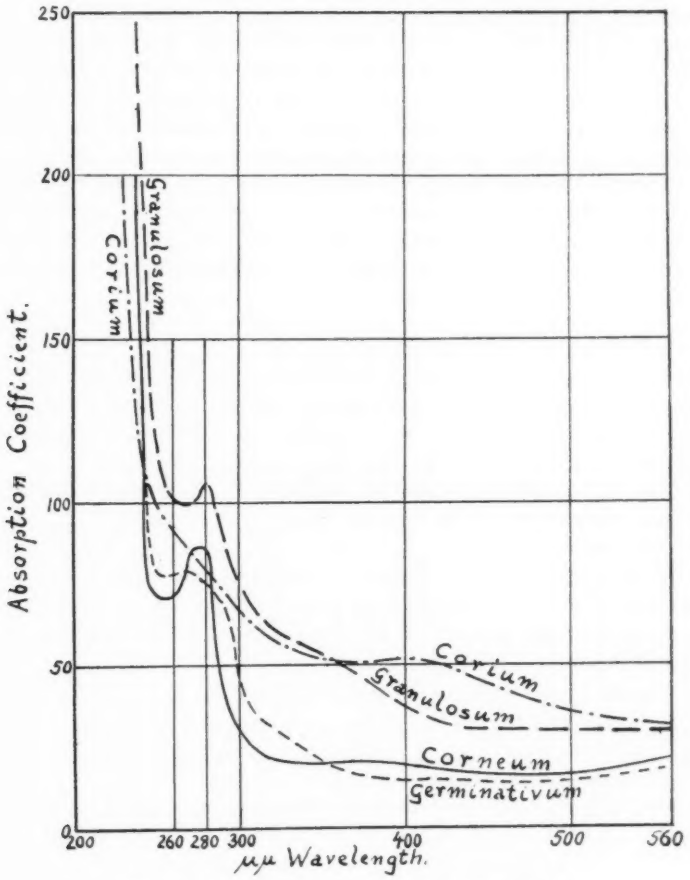

Fig. 4. Absorption curves for various skin layers

figures, as nearly as they could be obtained. A detailed investigation of the various constituents of this layer with a quartz microscope seems very advisable and is planned.

The stratum lucidum was obtained on two plates. The low absorption seems to extend over the whole ultra violet spectrum.

These observations lead to a few interesting conclusions: According to Sonne (2) the antirachitic effect is most pronounced at 280$\mu\mu$. This maximum coincides exactly with the absorption maximum of the stratum corneum and granulosum. This coincidence leads to the suggestion that the activation against rickets takes place within these or one of these two layers, probably by a physiologically active absorption. This observation excludes the possibility that this effect takes place in the germinal layer or in the corium, as these layers are particularly protected against these wave lengths by the higher layers. On the other hand the observations of Hausser and Vahle (3) show a sensitivity minimum for the erythema production at 280$\mu\mu$. The coincidence with the absorption maximum of the stratum corneum and granulosum indicates that this effect must originate in the lower layers of the skin, the germinativum or the corium, in the shadow of the upper layers. The explanation as given in a former publication (1) is entirely upheld by these investigations.

SUMMARY

By a new method of observation the following results have been obtained:

1. The exponential law of absorption does not hold accurately for various thicknesses of sections, on account of reflection, scattering and fluorescence (under certain conditions).

2. The skin cannot be considered as consisting of a series of layers with identical absorption. The various layers show pronounced absorption differences.

3. A comparison of the absorption curves of the various skin layers suggests that the antirachitic effect is produced in the stratum corneum or granulosum and that the erythema originates in the stratum germinativum or corium.

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UNIDIRECTIONAL BLOCK IN HEART MUSCLE

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Several very convenient terms have recently been added to physiological terminology by Schmitt and Erlanger (1928). Among these are *monodromia*, a state of the tissue "in which the impulse is transmitted in one direction and blocked in the other," and *heterodromia*, a condition in which the impulse is transmitted more rapidly in one than in the opposite direction. When conduction is *isodromic*, the velocity in the two directions is equal.

In the present communication we report a method which makes it possible to obtain a monodromia in a turtle ventricular strip and to determine, in the majority of cases, the direction in which blocking shall first supervene.

METHODS. We have produced heterodromia and monodromia by local compression. Instead of using a clamp, we compressed the muscle with the "compressor" illustrated in figure 1. A light glass tube, 26.0 cm. long and 1.27 cm. in outside diameter was fitted snugly, but not tightly, into two metal sleeves which were clamped to a stand. Attached to the upper end of the glass tube was a large flat cork surmounted by a cardboard box. A number 00 rubber stopper was inserted tightly into the lower end of the tube, and was cut, at its lower and broader end, so that when the glass rod stood vertically, the lower surface was a rectangular plane, measuring 12 by 8 mm., and forming an angle of about 10° with the horizontal (fig. 1B). The slope was from one wider edge to the other. Thus, when the sloping surface was placed transversely upon the ventricular strip, one edge of the region of the muscle in contact with the stopper was more strongly compressed than the other.

To start the experiment, a strip was prepared from the ventricle of a 7-inch *Emys blandingii* or from a 9-inch *Chelydra serpentina* (hearts T to X). Four transverse strips were usually obtained from the latter, one from *Emys*. The strips varied from about 3 to 6 cm. in length, 5 to 8 mm. in width and 2 to 3 mm. in thickness. The strip was stretched out, inner surface down, on filter paper over a flat, horizontal surface. Before beginning the compression, conductivity was tested by break shocks delivered by

hand, through a commutator key and platinum electrodes, to either end of the strip. These and subsequent responses were recorded by the string galvanometer, one contact being placed at each end of the muscle.

The compressor was then lowered upon the muscle and conductivity again tested. Stimuli were now applied alternately at either end of the strip, a 10 gram weight being added to the cardboard box after each response. After the first failure of the impulse to traverse the length of the strip, the stimulus was again applied to that side. Thereafter, until the onset of adromia, the stimuli were applied in pairs at either end, but

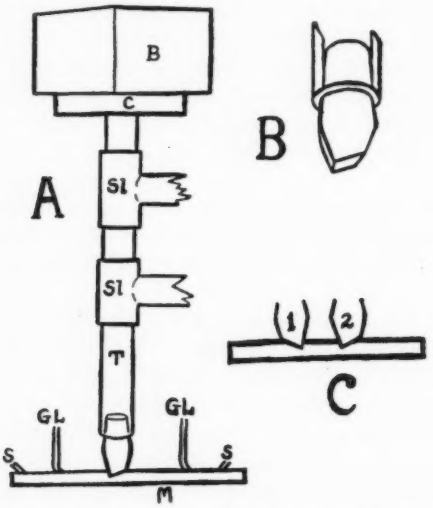

Fig. 1. Diagram of apparatus. *A*, the compressor; *Sl*, sleeve; *Gl*, galvanometer lead; *S*, stimulating electrodes; *M*, muscle. *B*. Enlarged lateral view of stopper in end of tube. *C*, *1* and *2* represent successive positions of stopper in certain experiments described in text.

there was always a 10 second rest between responses. Obviously this procedure was necessary to prevent the possible onset of 2:1 block from simulating a monodromia. In some of the early experiments this procedure was varied in that the stimuli were applied in pairs throughout. In the earlier experiments, too, there was no interruption in the addition of weights after each response; later the addition of weight was temporarily discontinued with the first failure to conduct and resumed after the other end of the muscle had been tested. With the onset of adromia, as indicated by at least two failures in the more favorable direction, stimulation was discontinued and the compressor lifted from the muscle. At short intervals

thereafter the muscle was tested for recovery from block, adequate care, of course, being exercised to avoid confusion between recovery through monodromia and heterodromia and the mere effect of a relatively longer rest.

In 18 of the longer strips, after recovery to the stage of a moderate heterodromia or, more commonly, isodromia, the orientation of the compressor was reversed and the muscle recompressed at a previously uncompressed region, the same procedure being again followed.

As has been said, our compressor squeezed the muscle most tightly at the lowermost edge of the inclined lower surface of the stopper and least at the opposite edge. It is possible that, as in Cattell and Edwards' (1928) experiments with nerve, there was relatively more injury at the boundary between the region most strongly compressed and the uncompressed muscle than elsewhere. The gross appearance of the muscle after compression suggested this, but it is probable that the effect was minimized by the flexibility of the rubber which, when subjected to pressure, yielded somewhat at the edge where the angle was slightly acute. There can be no doubt that a gradient in pressure was actually produced.

Choice of material and apparatus. Before undertaking this series of experiments we were very doubtful as to the practicability of the method. Given strips, perfectly homogeneous anatomically and physiologically, and a compressor cut at just the correct angle and of the right dimensions, we felt we should succeed. But neither anatomical nor physiological perfection is attainable, a fact which was only too apparent in a number of our strips. It would inevitably occasionally happen that undetected, preëxisting, moderate local injury in a strip would come into such relation to the compressed region as to offset or even reverse the effect of the compressor. It is to such inadvertencies that we attribute our few negative and some, at least, of our indifferent results. In addition to this source of trouble, it is improbable that the optimal angle or width of compressor was chosen. When we found that the first compressor made was yielding positive results, we used it throughout, fearing that any change might have been for the worse.

We wish to emphasize the fact that the data are presented for every test upon every ventricular strip used, with only five unavoidable omissions. These were cases in which, for one or another reason, no results, favorable or unfavorable to our findings, were obtained. In strips D and N intramuscular block unquestionably developed at a region other than that compressed. Strip V-4 developed block before the compressor was lowered. Strip O-1-b was recompressed, by mistake, at the original region of compression. If, in addition to these, we were to reject all results from experiments in which the electrograms suggest that partial or complete extra-compressional blocks had developed, we could reduce the number of

negative or indifferent results, particularly the adverse heterodromias. As we have said, however, this has not been done.

Probably the reader has already inquired why we did not produce heterodromia and monodromia by cooling. We did, in fact, undertake such experiments, both with ventricular and auricular strips, but in no case

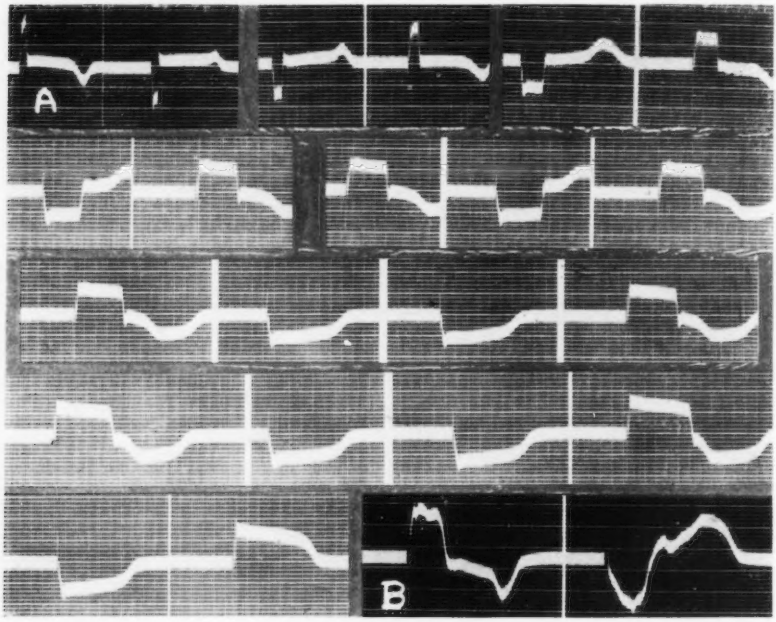

Fig. 2. A. Heart X, strip 1. Orientation of compressor such that conduction should first fail when stimulus is applied to right end of strip. In this experiment, as in all others, an initial upward deflection indicates stimulation of left, downward of right end of strip.

Line 1, first pair of responses, 50 grams compression; second pair, 150 grams; third pair, 350 grams. Line 2, first pair, 550 grams; next three responses 570 to 590 grams, shortly before onset of monodromia. Line 3, monodromia from 620 to 650 grams. Line 4, just before onset of adromia, 780 to 810 grams. Line 5, first pair, adromia, 840 to 850 grams.

B. Heart U, strip 2. See text.

could we demonstrate a consistent heterodromia or monodromia. The usual result was essentially an isodromia or partial or complete block without monodromia. Our failure was possibly due to our inability to establish a proper gradient; but the mere fact that the effect of cooling could not be sufficiently localized appears to be an adequate reason. There was too

much conduction of heat from outlying muscle regions. For reasons which will be apparent later, we did not attempt to employ chemical depressants. The technique used on skeletal muscle by Engelmann, as we shall show, appears to us inadequate for the demonstration of a true monodromia.

After completing the series of experiments herein described, hetero- and monodromias were obtained from 10 other strips. This was done by making two oblique incisions at opposite points on the sides of the strips. Thus one half of the strip, as it approached the center, was wedge-shaped, the apex of the wedge serving as a bridge connecting it with the other half. In 7 of the ten strips, monodromias, partial blocks with the higher grade of block in one direction, and heterodromias were obtained, the direction of more difficult conduction being clearly from the wedge-shaped to the other half. In 2 strips, the opposite result was obtained, although the difference in one case was not great, while in one conduction was equally free in the two directions.

These experiments, by themselves, are insufficient to demonstrate the relationships of depressed regions or of conducting muscle masses responsible for heterodromia and monodromia, but they are strongly suggestive when taken in conjunction with the experiments employing compression. They were particularly valuable, however, in that they enabled us to show even more definitely than in the other series of experiments, that the distance the impulse travels before arrival at the region of block is not the factor responsible for hetero- or monodromia.

Because the onset of very warm weather caused the early appearance of treppe phenomena in conduction (Ashman, 1925) and more rapid deterioration of the strips, we will carry this series of experiments no further at present.

Results. The impulse was initiated in uncompressed muscle near the end of the strip and at a distance from the compressed region. If, upon reaching that region, it was not blocked, it then passed the compressed and entered and traversed the uncompressed muscle at the other end of the strip. In the majority of experiments, when the pressure reached a critical value, conduction failed when the impulse was required to travel in the direction from lighter to stronger pressure. In the typical case it was only after the addition of more weight that block also supervened in the opposite direction. It is worth noting, perhaps, that in most cases the conduction time for the last impulse transmitted in the direction from less to greater pressure was shorter than for the later last impulse transmitted in the other direction. This latter impulse was, of course, transmitted after, and sometimes long after the onset of block in the other direction. A similar fact, i.e., that failure of conduction bears no constant relation to conduction time just before failure, has been observed by Garrey.

After calling attention to the criteria upon which one must base a decision

as to the effect produced by the compression, we will describe our results in more detail. The criteria in question are four. The first and by far the most unambiguous and trustworthy, is the onset of a monodromia during the period when the pressure is increasing. The second criterion, third in importance and less consistent, is the appearance of a heterodromia just before the onset of block. The third, of second importance, is the occurrence of monodromia during recovery from compression, and the fourth, the presence of heterodromia after further recovery. It is necessary to note that, with over-compression, recovery sometimes failed; that in some cases, probably because of too long a rest between tests, we failed to record the stage of monodromia during recovery; and that, in other cases, recovery to the stage of monodromia was alone recorded. Our data on recovery are, therefore, incomplete.

If, for the above reason, we take monodromia or heterodromia during recovery as a single criterion, giving a total of three, it may at once be said that in 16 of the total of 53 trials, *all* the recorded criteria were *positive*, i.e., of a character clearly related to the orientation of the compressor. In only 1 case were they all negative.

Monodromia during induction of block. Those portions of figure 3A blocked in in solid black, will convey a clearer conception of the monodromias in our experiments than could be obtained from the casual examination of a table. In this figure the successive trials have been arranged in chronological sequence. The capital letters below represent individual turtle hearts. The numbers, just below the letters, are the strips cut from these hearts. The small letters, *a* and *b*, on a still lower line, indicate that, for the strips in question, the orientation of the compressor was reversed and thus two tests were made on the same strip. The height of the black column over each number or letter indicates the weight added between the appearance of monodromia and of adromia expressed as the percentage of the total weight necessary to produce adromia. This percentage is at least as great as represented. Because of the exigencies of the experimental conditions, the actual weight could often have been estimated as from 10 to 20 grams more than this minimal. When the column extends downward the significance is the same, but the direction of the monodromia is opposite to the usual, and we therefore speak of the result as *negative*. Where question marks are placed, the percentage was, for one reason or another, impossible to determine. The height of the column in these cases is, therefore, set at the average. The small white squares have to do with recovery and their significance is described under that heading.

It will be seen that in 30 of the 53 tests, there was a positive monodromia, clean cut and definite excepting in 1 or 2 cases. In 21 tests no monodromia was demonstrable during induction of block. In 1 of these trials, no block was produced. In 2 tests only was there a negative monodromia, i.e., in a direction opposite to that usually induced by the compressor.

The pressure required to produce monodromia was variable. In one strip (I-b) it resulted from the weight of the compressor alone (50 grams). In another trial (W-1-a) all the weight that could be placed in the box (1170

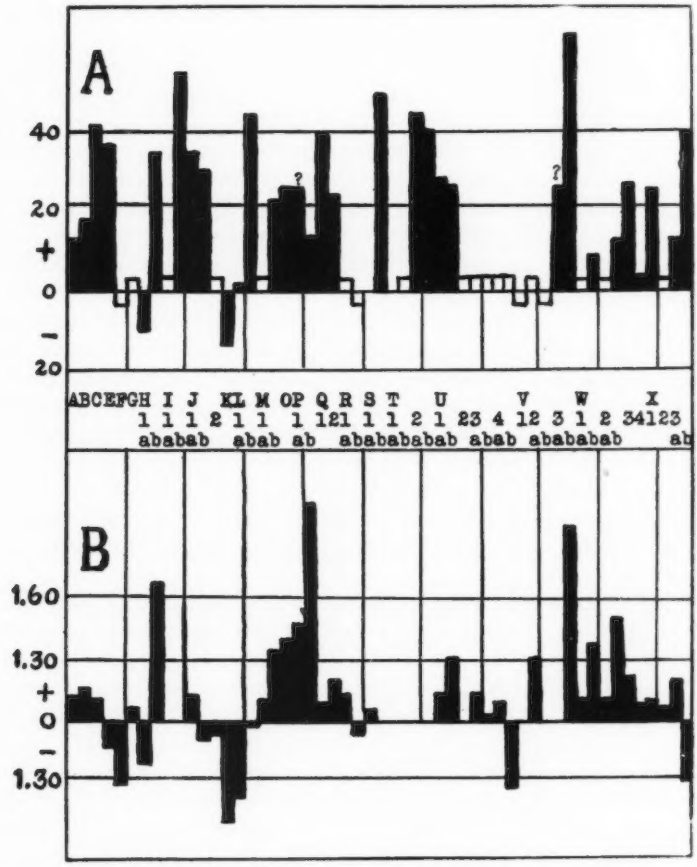

Fig. 3. A, monodromia during onset of block; B, heterodromia during onset of block.

grams) failed to induce block. When the compressor was reversed on this same strip, 870 grams were required to produce monodromia. The average weight needed in the experiments was nearly 200 grams. In general, the load varied with the condition and with the width and thickness of the strip, more being required for wider and thicker strips.

Heterodromia during induction of block. In general heterodromia has been determined from the conduction times in the two directions just before the onset of monodromia. In most cases this was easy to determine, but in many experiments difficulties arose. These latter, illustrated in figure 2B, were chiefly due to two causes: a, the occasional occurrence of slow conduction between the stimulating electrode and the nearer galvanometer lead, an event which sometimes rendered uncertain the time of response just below the latter electrode, and b, a difference in the promptness of the fall in potential under one electrode depending upon the direction in which the impulse was moving. However, most of the responses, as in figure 2 A, were definite. Our negative heterodromias are probably in part due to uncertainties in measurement, in part to spontaneously developing heterodromia outside the compressed region and opposing the heterodromia in the latter, and in part to inequalities in the physiological condition of the compressed muscle itself.

Our method of measurement was essentially as follows. The last conduction time before onset of monodromia or adromia was averaged with the preceding time in the same direction. The conduction time in the other direction for the response interpolated between the two just mentioned was also determined. In every case, from these two quantities, a conduction quotient was calculated by dividing the longer by the shorter time. Thus, as represented in figure 3 B, a positive quotient of 1.20 means that conduction time was 20 per cent slower in the direction in which block was first expected than in the other direction. A negative quotient means just the reverse.

We have no heterodromias for turtle T because we found, after preparing the heart, that our source of constant current for the galvonometer was not available. The other omissions are for experiments in which it was impossible to be reasonably sure of the conduction times. But there is no reason to believe that a higher percentage of these would have been negative than among those measured. After such omissions we have a total of 42 determinations of heterodromia of which 31 are positive and 11, negative. This is a higher percentage of negative findings than for any other criterion.

Returning to the 30 examples of positive monodromia in our 53 tests, heterodromia was measureable in 25. In 20 of these the heterodromia was positive; in 5, negative. Of the 21 cases in which a definite monodromia failed to appear, heterodromia was determinable in 15. Of these, 11 were positive, 4 negative. There were only 2 negative monodromias and in each of these heterodromia was negative also. The higher percentage of positive heterodromias occurred, therefore, when monodromia was also positive.

One other phenomenon, which may account, in part at least, for the variable differences in conduction times in the two directions, should be

noted here. Generally, the conduction time in each direction was distinctly prolonged as soon as the muscle was made to support the weight of the compressor. After the addition of each increment in weight there was a further increase until the conduction intervals were several times as long as in the uncompressed muscle. Then, more or less abruptly, conduction failed in one direction. Sometimes, just before failure, there was an apparently greatly accelerated increase in conduction time in that direction, suggesting that at a certain critical point the changes leading to depression

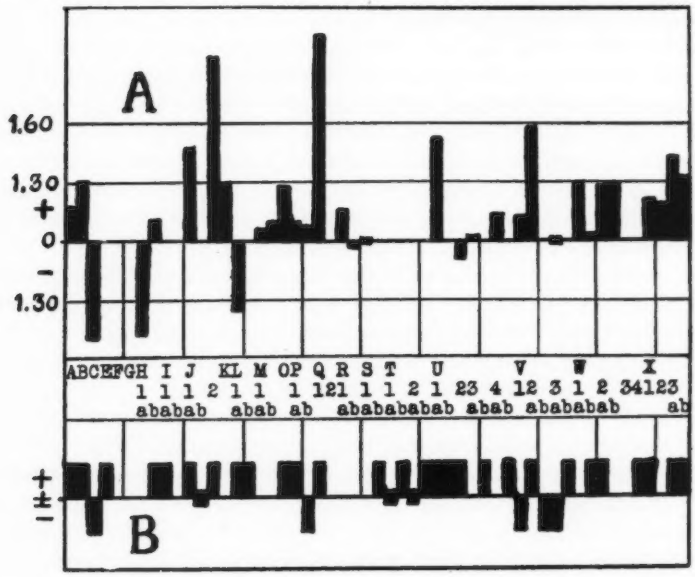

Fig. 4. A, heterodromias during recovery from block; B, monodromias during recovery from block. Since obviously there was no basis for determining percentage values, the figure indicates simply that the individual experiment was positive, indifferent or negative.

of conduction were more rapid. Because of the rapidity of this change and the impossibility of testing conduction in both directions simultaneously or at frequent intervals, the longest possible conduction time just before block was probably rarely, if ever, recorded. Had it been recorded the heterodromia would undoubtedly have been more striking. It is not impossible, in fact, that this factor, i.e., the rapid transition from slow conduction to block in the one direction, sometimes prevented the detection of a heterodromia which, so far as the influence of the compressor was concerned, was positive rather than negative. In such cases the hetero-

dromia during recovery should demonstrate the true effect of the compressor. In this connection the following data are of interest. In 6 of the 11 instances of negative heterodromia during the onset of block, heterodromia was also recorded during recovery, and of these 6, 3 were very clearly positive, while in 1 other case the negative heterodromia had almost disappeared. For comparison with this reversal of direction, it is of interest to note that in the 24 cases in which a positive heterodromia occurred during onset of block and in which heterodromia was also determinable during recovery, only 1 of the latter was negative. And this one exception was in a case of greatly delayed recovery.

Heterodromia during recovery from block. As we have already noted, complete block was persistent in a number of cases, while recovery only to the stage of monodromia was recorded in others. With 2 exceptions, when recovery was obtained, not more than 10 or 15 minutes were needed after release from compression. The time was usually less. In one of the exceptional cases (strip C) recovery was delayed for over 30 minutes and, in spite of positive heterodromia and monodromia during induction of block, these were negative during recovery.

The heterodromias determined during recovery are available for 33 tests. Of these 26 are positive, 2 are indifferent and 5 are negative (fig. 4A). Perhaps because the strips whose condition was best yielded the highest number of recoveries from block, our results are here more positive than for heterodromia during the onset of block.

Reverting to the 30 cases of positive monodromia during the onset of block, we find that in only 18 was heterodromia determined during recovery, but of these, 15 were definitely positive, one being indifferent. Only 12 heterodromias were obtained during recovery in the 21 tests in which monodromia had been indifferent. Of these 12, 9 were positive, 1 was indifferent, and 2 were negative. During the recovery of the 2 strips which had shown a negative monodromia, in 1 heterodromia was also negative; in the other, positive.

Monodromia during recovery from adromia. This stage of recovery was recorded in 36 of the 53 tests. Of the 30 cases of positive monodromia occurring with the onset of block, monodromia was recorded during recovery in 25 cases. Of these 25, 20 were positive, 2 indifferent and 3 negative. Among the 21 instances of no monodromia during induction of block, 8 showed a positive monodromia during recovery, 1 was indifferent, and 2 were negative. In the other 10 cases this stage of recovery was either unrecorded or there was no recovery. In the 2 cases showing a negative monodromia during onset of block, this stage of recovery was not recorded.

Character of the effect produced by the compression. A slight degree of monodromia, varying in different hearts from 1 or 2 to as much as 10 per cent, would generally have escaped detection because of the rapid transition

from slow conduction to block. There can, therefore, be little doubt that many of our indifferent results would have proved to be either positive or negative if a refinement of technique had been possible. Fortunately we possess, in most of these cases, criteria from which the probable effect of the compression can be determined. These criteria are the heterodromia during the onset of block and the monodromia and heterodromia during recovery. Reasons have already been given why we regard the monodromia as the more significant, but, in the absence of monodromia, the heterodromia affords a good, though less certain, indication.

We may now proceed to a further analysis of our results in an effort to separate those which are predominantly positive from those which are predominantly negative. In 11 trials out of the 53, we recorded both positive monodromia and heterodromia during the onset of and recovery from block. In 5 other cases, hetero- and monodromia were both positive during the induction of block, and either heterodromia or monodromia during recovery, the other being undetermined. In addition to these, in 5 cases no recorded criterion was either negative or indifferent. These 21 trials may, therefore, be taken as definitely positive without further discussion. Of the remaining 9 cases among the 30 showing positive monodromia with induction of block, none are negative with respect to all the other recorded criteria, and only 1 can be regarded as indifferent in so far as the total effect of the compression is concerned.

Of our 21 cases showing no monodromia with onset of block, 13 are almost certainly positive with respect to the effect of the compressor; 3 are most probably positive; 1 is indifferent; 1 is probably negative and 3 are almost certainly negative.

The single case in which the compression failed to produce a block is included among the above and should certainly be counted as positive; while the 2 examples of negative monodromia with onset of block are predominantly negative.

Thus when the total effect of the compression is determined on the basis of an analysis of all the available criteria, we find that 44 of the 53 examples should be regarded as predominantly positive, 3 as indifferent, and 6 as predominantly negative. This classification of the results is illustrated by the white squares and black areas in figure 3 A.

Even more convincing than the data already adduced are the results of those 18 experiments in which the orientation of the compressor was reversed on the same strip. In 7 of these cases, the monodromias in both directions were positive. If, instead of considering only monodromias, we take the total effect of the compression, then definite reversal in the expected sense was produced in 13 cases. In 1 case, reversal of the compressor apparently changed a moderately negative to a strongly positive result; while in 2 cases an initial positive was not fully reversed. In two cases, the initial effect was indifferent, but positive after reorienting the compressor.

From the foregoing description of results it will have been seen that, in 83 per cent of the tests of the effect of the compressor, the evidence demonstrates that conduction is easier when the impulse is required to traverse the muscle in the direction from stronger to weaker compression. In only 11.3 per cent of the tests does the evidence point in the opposite direction. These results are not due to misinterpretation, as, for example, the onset of a 2:1 block, nor are they possibly due to a systematic error of any kind, since the direction of monodromia was reversed on the same strip in several instances, and the orientation, with reference to the stimulating electrodes used, was changed from strip to strip. There is, in fact, only one conceivable source of error. If, as was not impossible, any of our strips were in such poor physiological condition as to conduct with a decrement when uncompressed, then the distance the impulse had to travel before reaching the region of strongest compression might have been a factor in determining the direction of the monodromia (Schmitt, 1928). That this was not the factor is, however, proved by the following. In several cases the orientation of the compressor was as illustrated in figure 1C in the two tests on the same strip. Thus, for both trials, the impulse traveled farther to the compressor in the direction of longer continuing conduction than in the other direction. Furthermore, for the second trial, the impulse was compelled to pass the original region of compression before reaching the compressor, a region in which there was never complete recovery. And yet, in spite of this retardation of the impulse at a distance of but 0.5 to 0.7 cm. from the compressor, the impulse continued to pass in this direction after failure from the other end.

DISCUSSION AND CONCLUSIONS. Irreciprocal conduction or undirectional block has received attention not only because of its spontaneous appearance at the A-V junction in the heart (Engelmann, 1894; Mines, 1913; Skramlik, 1920) and its probable importance in the initiation of fibrillation or flutter (Garrey, 1914; Schmitt and Erlanger, 1928) but also because of its normal occurrence at synapses and at myoneural junctions. This was emphasized by Engelmann (1895).

As a result of his work upon skeletal muscle, Engelmann (1895) formulated the general rule that conduction is easier from a region of high to one of low conductivity than in the reverse direction. However, Schmitt (1928) says of his own results with turtle ventricular strips that they "fail to clearly support" this conclusion of Engelmann. Our results, as the reader will have seen, are in agreement with those of Engelmann. Before attempting to explain the phenomenon of irreciprocal conduction, it will be necessary briefly to review the explanations which have hitherto been proposed. In doing this it will be unnecessary to review modern conceptions of conduction, or the evidence that, whatever may be true for the nerve fiber, there is a growing body of evidence that conduction with a decrement,

perhaps transitional (Davis, Forbes, et al., 1926), is found in depressed or relatively refractory cardiac muscle (Drury, 1925; Drury and Andrus, 1924; Schmitt, 1928; Drury and Regnier, 1928). It may be emphasized here that the explanation of our results is not contingent upon, although not in conflict with, the occurrence of conduction with a decrement.

Mines (1915) suggested that "the cause of unidirectional block may very likely be expressed thus in terms of Adrian's work. . . . If the decrement is uniform, then the system is symmetrical and the block should be equal

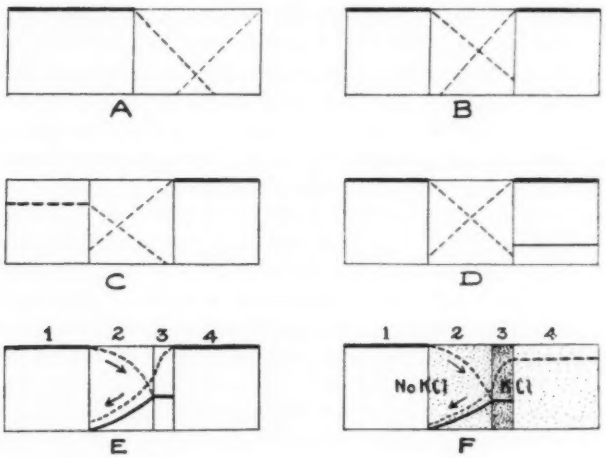

Fig. 5. Schema slightly modified from Schmitt and Erlanger. A. An impulse from left end of strip enters depressed region and decrements to extinction along dotted line. See text. B. Significance of heavy and of dotted lines the same as in A. The impulse from left is not extinguished and causes response of right end of strip. C. The impulse from left is of smaller initial magnitude and decrements to extinction in depressed region. D. The threshold of right end of strip is raised so that it fails to respond to impulse from left. E and F, heavy line in segments 2 and 3 represents raised thresholds. These latter diagrams can not adequately express our conception of the nature of the monodromia. For fuller explanation see text.

in the two directions. But if the decrement is greater at one end of the depressed region than at the other, we may have the possibility that the transmission in the one direction may be easier than in the other." This brief description of the conditions which he thought might be responsible for the phenomenon seems to us to be open to two interpretations. One has been made and illustrated by Schmitt and Erlanger (1928), and in figure 5B we reproduce their diagram representing Mines' view. From Mines' phraseology, however, it is not at all certain that he did not have in mind a condition in the tissue somewhat similar to that we have produced. The

assumption that the decrement in one direction through compressed muscle is greater than in the other, while perhaps true, does not show how such a differential decrement can come about, and, to that extent, falls short of a true explanation.

Schmitt and Erlanger's own conception of the factors involved is illustrated in figure 5C and D. Assuming an equal decrement in both directions in that region where block may occur, they show that if the muscle beyond the block at one end of the strip possesses a lower degree of conductivity than the other end, an impulse set up in that end may fail to pass the region of decrement because it is already weak when it reaches that region (fig. 5C). On the other hand an impulse from the other end may pass. They also present the alternative suggestion, that with equal conductivity at the two ends of the strip, the excitability at one end may be lower. Thus the impulse arriving at this end after undergoing decrement in the depressed region will be too weak to excite (fig. 5D). The apparent difficulty with the second suggestion is that it is hard to conceive of a region of muscle whose excitability is depressed in which conductivity is not equally depressed. It is, therefore, not clear, as they assume, that the impulse initiated in the end whose excitability is low will be sufficiently strong to pass the region of decrement. Regarding their first suggestion the case is possibly somewhat different, although it involves the same difficulty mentioned below in our discussion of Engelmann's results. As they have shown, when the impulse arriving at a region of decrement is already weakened, it may actually undergo a sharper or more rapid decrement than an impulse of normal magnitude. Thus it fails to pass. The impulse from the other end of the muscle, being of greater strength, will at first travel with a less sharp decrement, and, arriving at the slightly depressed region beyond, may still be an effective stimulus. However, in about 60 per cent of the cases in which excitabilities were tested by Schmitt and Erlanger, the freer conduction was in the theoretically wrong direction.

The experiments of Engelmann with skeletal muscle were carried out under conditions which make an interpretation of the results difficult. There was no middle region of lessened conductivity. The depression was of one end of the muscle. It is thus not impossible to explain his results as in figure 5 A. An impulse starting from the depressed end of the muscle, may have caused a response of the greater part of that end and yet not have reached the boundary between it and the untreated end. The impulse from the latter end, on the other hand, may have penetrated the depressed muscle sufficiently to have yielded a recordable response, yet not have fully traversed that region. The phenomenon thus becomes one of decrement, equal in both directions, and incompetent to explain a true monodromia or heterodromia.

We may now proceed to an interpretation of our own results. As we

see it, the essential condition for the production of a monodromia is the presence of an asymmetric region of injury or depression such as we have produced (fig. 5 E). Thus the impulse, set up in normal muscle and traveling from the left, first encounters a region of moderately depressed excitability and is so reduced as to become an ineffective stimulus for the more depressed muscle immediately beyond. And furthermore, the impulse is slowed or weakened to such an extent that the action current, acting at a distance beyond the region of greatest compression, is not an effective stimulus of the normal muscle at the right end. On the other hand, the impulse arriving at the asymmetrically compressed segment from the other direction, is effective in causing a response of the more depressed part. The action current of the latter, together with the current from the normal muscle acting at a distance, suffices to cause the less depressed segment to respond. The latter, in turn, passes the impulse on to the normal muscle.

The crux of the interpretation is the presence of a segment, responding to impulses in the one but not in the other direction, whose liberated energy makes transmission possible in the one direction.

It is easy to choose arbitrary numerical values for the excitability of each region concerned, and for the effectiveness of the action current in each segment acting as a stimulus to immediately adjacent and more remote regions. For simplicity we assume that we are dealing with four segments of muscle: normal, less depressed, more depressed, normal. The stimulating effectiveness of the action current from each segment, from left to right in figure 5 E, may be expressed respectively as 100, 50, 14.3, 100. The stimulating effectiveness at a distance, e.g., of the first segment on the third or of the second on the fourth may be regarded as one-tenth of the values of the active segments. The actual effectiveness will of course depend largely upon the width of the intervening segment. The thresholds for excitation are taken as inversely proportional to the first set of values, i.e., 10, 20, 70, 10. Now as the impulse travels from the left, the second segment will, of course, respond to the first. The third will be acted upon by the 50 units of stimulating current from segment 2, and by the 10 units extending from segment 1, a total of 60 units. Since the threshold of segment 3 is 70, this is ineffective and it does not respond. Furthermore, the 5 units of current from segment 2 acting upon 4 is insufficient. Thus blocking occurs. On the other hand, when the impulse travels from right to left, segment 3, with a threshold of 70, responds to the 100 units from 4. The 14 units from segment 3 together with the 10 units from 4, is more than enough to excite segment 2, which in turn excites segment 1.

This schema applies equally well if a series of depressed segments be postulated to lie between the two ends of the muscle, providing a proper and sufficient degree of asymmetry exists.

Just before the appearance of monodromia, the impulse from left

to right will be of just threshold strength and there will be considerable delay in conduction. From right to left, however, the stimulating action currents will definitely exceed the threshold values and delay will be less pronounced. Heterodromia may be thus explained.

The advantage of this interpretation is that the depression of a segment which causes a rise in its threshold to excitation, is regarded as producing a corresponding and equivalent decrease in the stimulating effectiveness of its action current. Whether this is strictly true we have no means of determining. In view of our ignorance on this point, however, it is better to make the more probable assumption that the depression is equal and to avoid the extremely unlikely view that a region whose excitability is depressed may, nevertheless, conduct an impulse of normal velocity and stimulating strength.

If we adhere strictly to the view that the excitability of a segment and the stimulating effectiveness of its action current are equally reduced by compression, we can not then logically assume that the most compressed segment fails to respond to the impulses in each direction and that the impulse jumps the gap in the one but not in the other direction. The difficulty in such an assumption would be the same as that which we have already discussed in connection with Schmitt and Erlanger's schemata.

The explanation here offered for monodromia may be looked at from a different angle. It is highly improbable, considering the character of the compression produced, that, when monodromia was present, the whole transverse width of the strongly compressed segment was responsive. It is much more probable that only a relatively small group of fibers were responding. Thus the preparation probably consisted of the two relatively normal uncompressed ends connected by a narrow bridge of responsive fibers. This bridge, more or less irregularly perhaps, would become narrower as it passed from the region of weaker to that of stronger compression. It is, therefore, not impossible that the relative masses of responsive muscle at either end of and within the bridge was a factor determining the direction of the monodromia. The interpretation we offer will apply in this case also.

It now becomes necessary to return to Schmitt and Erlanger's results. In their series of experiments on monodromia and heterodromia, they used long strips from the turtle ventricle. These strips were passed through holes in a series of several rubber membranes. Thus each successive segment of muscle was made to lie in a solution-tight chamber. Each membrane, which served as a partition between chambers, produced a variable degree of compression and frequently, at least, slowing of conduction occurred at these points. When Ringer's solution was introduced into the chamber or chambers at one or both ends of the strip and Ringer's containing an excess of KCl in the others, monodromia or heterodromia often

appeared between the differently treated segments. In 44 cases, the excitability of the muscle segments was tested on the two sides of the membrane. In 27 trials, conduction was more rapid from the segment of lower to that of higher excitability; in 8 trials the reverse was the case; while in 9 trials the results were not definitely in the one group or the other. Schmitt and Erlanger were inclined, because of the lack of consistency, to regard these findings as of no significance excepting in so far as they tended to show that some factor other than the effect of the solution was responsible for the hetero- or monodromia. This factor, they tentatively suggest, was a preexisting tendency of certain groups of muscle fibers to conduct more easily in one direction than in the other.

Although not conclusive, we believe that their 27 results of one type against but 8 of the other make it highly probable that the treatment of the muscle did play an important part in determining the direction of the hetero- or monodromia. And, in view of the fact that their results are, at first sight, apparently directly contrary to those of Engelmann and to our own, we feel justified in attempting an interpretation. Schmitt and Erlanger quote the work of Clark (1913) which demonstrated that hypodynamic muscle is more susceptible to the effect of depressant substances than normal muscle. As Schmitt and Erlanger say, a certain degree of depression was produced by the pressure of the partitioning membranes. That part of the muscle, as they recognized, was, therefore, probably more profoundly depressed by the excess potassium than the segment between the partitions. We may, perhaps, represent the conditions as in figure 5 F, the depth of the shading representing the relative degrees of depression. It will, if our assumptions are right, at once be evident that the impulse passing from the segment exposed to potassium first reaches the most depressed region of the muscle. It may be assumed that it has suffered no appreciable decrement. Therefore, as in our experiments, it causes a response of the greatly depressed region and consequently passes through to the muscle beyond. An impulse coming in the opposite direction, however, is first slowed by the slighly compressed region whose excitability is next to the lowest. The slow, and therefore less effective impulse, cannot stimulate the most depressed region beyond, and blocking occurs.

If this visualization of the conditions approximates the truth, then the type of asymmetry and the fundamental cause of the monodromia in Schmitt and Erlanger's and in our experiments is the same.

It does not necessarily follow from our experiments that all monodromia and heterodromia is a consequence of the type of asymmetry which we produced by means of our compressor, although we suspect that those phenomena are due to analogous or similar conditions. Nor do our experiments prove that preëxisting heterodromic or monodromic pathways are not present in ventricular muscle, although, again, we believe that their

presence is extremely doubtful. Our results also suggest, we believe, that experiments indicating that certain bundles of the A-V funnel of the cold blooded heart conduct more freely in the one than in the other direction should be reëxamined to determine whether an asymmetry, either in successive conducting elements or in muscle masses may not account for the findings (Skramlik, 1920).

SUMMARY

By means of asymmetric pressure applied to the middle of a ventricular strip from the turtle heart, it is possible, in the greater number of cases, to produce irreciprocal conduction in the direction desired.

An impulse passing from normal to a narrow region of strongly compressed muscle may pass on to a less strongly compressed region and thence into normal muscle. If the impulse is set up at the opposite end of the strip, so that it must traverse the less before reaching the more strongly compressed region, it may fail to pass the region of compression.

Under similar conditions, before irreciprocal conduction appears, the impulse will usually traverse the strip more rapidly in the direction from stronger to weaker compression than in the opposite direction.

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POLARIZATION-CAPACITY AS MEASURED WITH A WHEATSTONE BRIDGE WITH SINE-WAVE ALTERNATING CURRENTS OF HIGH AND LOW FREQUENCY

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Studies of polarization-capacity at electrode surfaces or living membranes have usually been made by measuring impedance to the electric current by substitution-methods. The Wheatstone-bridge has the advantage that the two components of impedance, namely, resistance and capacity-reactance may be distinguished. On the other hand the Wheatstone-bridge-method is extremely difficult at high frequencies. Mica condensers heat up at high frequencies and consequently do not represent pure capacities. Resistance-units show inductance and distributed capacity and in the placing of these units to form a bridge-circuit, there is distributed capacity between the elements themselves or, if shielded, between the elements and the shields. The latter difficulty may be partially obviated by means of the Wagner-ground but the operation of this ground is sometimes very tedious as it means re-setting of the ground every time any of the switches of the bridge are operated. It therefore seemed desirable to build an accurate bridge for high frequencies. The building of this bridge has taken about 10 years and it is not yet perfect, but it has many points of superiority which may be noted briefly. Figure 1 shows a diagram of the apparatus. *A* is an oscillator designed for stability of frequency similar to the one used by Stratton. Being a twin-oscillator the grid of one electron-tube is connected with the oscillating circuit of the second and the grid of the second connected with the oscillating circuit of the first. Furthermore, in each plate-circuit there is a very high resistance. To secure further stability the oscillator is inductively connected very loosely with the bridge. The ratio-arms of the bridge proper are denoted by *KK*. They are as nearly pure resistances and equal as it was possible to make them after several years of selection. Each ratio arm *K* consists of a thin film of highly resistant material on the outer surface of a tube of

"isolantite." The ends of the film are copper-plated and surrounded by a tight-fitting metallic band to insure electric-contact. The resistance of

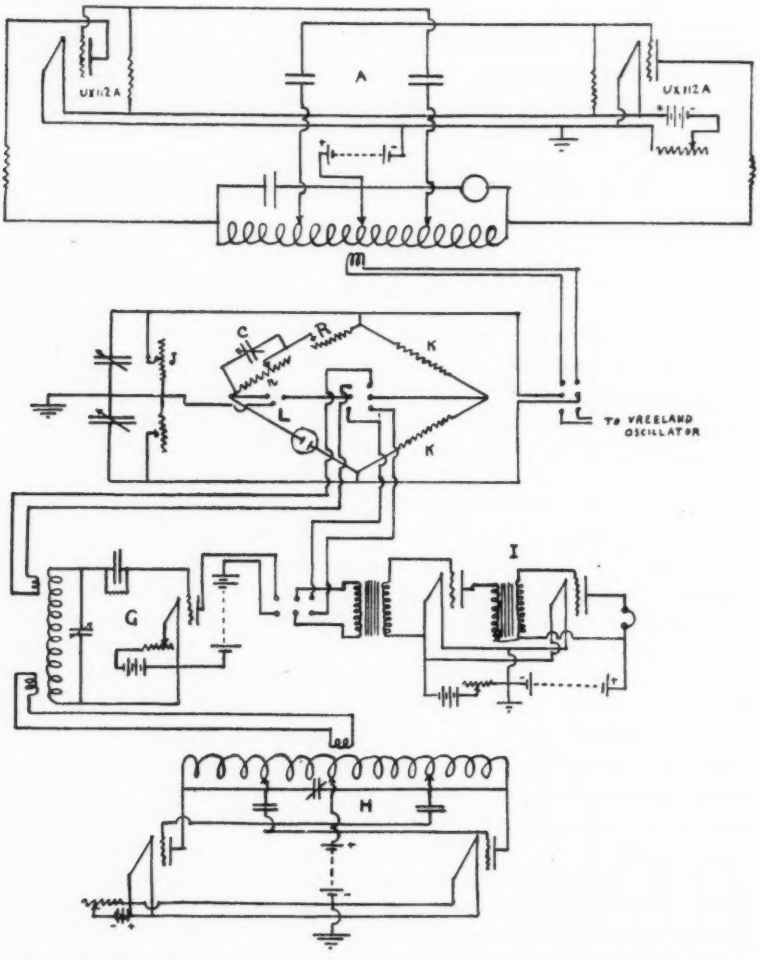

Fig. 1. Wheatstone bridge for measuring polarization-capacity of electrolytes with currents of high and low frequency.

one K is adjusted equal to that of the other K by scratching off part of the film in a circular direction, i.e., not in a spiral direction. The ratio-arms are so arranged that they may be quickly interchanged to verify their equality.

One of the arms of the bridge contains a variable resistance R in series with a variable capacity C which is in parallel with a variable resistance r. The resistance r has five decades made of units similar to K except the small units which are wound like the large Curtis coils, that is to say, a figure-eight winding. Instead, however, of using a slotted cylinder to wind the coil a slotted plate was used. The resistance R is similar to r. The decade condenser C consists of three large mica decades made by Leeds and Northrup, a decade of fixed-air-condensers made with plates 0.1 mm. apart and a large variable air condenser for the final adjustment. The variable air condenser was very accurately calibrated as were also the fixed condensers. The conductivity-vessel was placed below L in figure 1 to make up the fourth arm of the bridge. A Wagner-ground represented at J consisted of decade resistances and variable air-condensers. The detector is is shown at G, the heterodyne oscillator at H, and the two-stage amplifier at I. The amplifier works best at 1000 cycles and so the heterodyne oscillator was tuned to give the beat-note of 1000 cycles which was rectified by the detector and heard in the telephone connected with the amplifier. Low-frequency-oscillations were generated by a Vreeland-oscillator capable of a wide range of frequencies. A General-Radio-Company precision-wavemeter was used to determine the frequency. A great deal of time was spent in carefully shielding the apparatus. Absolutely every part of the apparatus, except the telephone-receivers and the Vreeland-oscillator, was shielded in copper-shielding spaced about four inches from the conductors, and each resistance or capacity was placed in a separate shielded compartment. The wires connecting one unit to another were supported on tall insulators and passed through one inch holes in the shield to the next compartment. No insulation or other solid matter was used in these holes but the wires were entirely free from the shield, that is to say, suspended in air. The shield in which the conductivity-vessel was placed contained an electro-thermo-regulating apparatus that kept it at $25° \pm 0.1°$. At the moment of verification of the reading, the thermo-regulating apparatus was temporarily thrown out of action by a multi-pole double-throw switch which grounded every part of the thermo-regulating apparatus. The inductance-couplings between the oscillator and the bridge, the bridge and the detector, the detector and the heterodyne, and the detector and the amplifier were protected by electrostatic shielding which was perforated by parallel slots in such a way as to allow inductance-coupling but secure adequate electrostatic shielding. The conductivity vessels were of two types and will be described in relation to the measurements.

It was shown by Warburg (1901), Wolff (1926), McClendon (1927), and others that polarization-capacity varies with voltage and also with frequency, and with the concentration of the electrolyte. This may be pictured in the following manner: Electricity is carried through the conduc-

tor of the first class, i.e., metal, by means of the migration of free electrons in a potential-gradient but the resistance of the metal to the passage of the electrons is so low that the potential-gradient need be but very small, in fact, for our purpose we may neglect it. By using copper and gold conductors of considerable size we may neglect their resistance. Electricity is carried through the metal by free electrons with charges all of the same sign and which would repel one another and tend to be distributed widely (although not equally) when the current is not flowing. On the other hand, in the electrolyte the current is carried by ions of two classes, positive and negative. Two ions of opposite sign should attract one another and neutralize the charges. The fact that they do not indicates that they are not entirely free. If an ion is surrounded by a shell of water (hydrated) these shells of water may bump into one another but the electric charges do not come into contact ordinarily. If a piece of metal is placed in the electrolyte-solution it behaves to some extent like a polyva-

TABLE 1

f	C	
	Observed	Calculated
	μF	μF
500	0.755	0.728
1,000	0.631	0.631
1,500	0.580	0.580
2,000	0.545	0.546
2,500	0.523	0.522
3,000	0.504	0.501
3,500	0.490	0.489

lent ion of large size, that is to say, it is surrounded by a shell of water so that the ions in the solution do not collide with the metal but the water-shells of the ions bump into the water-shell around the metal. If we place two metal electrodes in an electrolytic solution and charge one positively and the other negatively, the positive electrode attracts an excess of negative ions to its proximity and thus forms, with this ionic layer, a condenser. The surface of the metal is charged positively thus acting as one plate, the army of negative ions acting as the other plate of a condenser. By diffusion the ions tend to redistribute but the difference of potential between the metal and the solution tends to draw them back and the greater the potential-difference between the metal and the solution the nearer will the negative ions be drawn to the metal. At the other electrode a similar process takes place but with a reversal of signs. The surface of the metal is charged negatively and attracts positive ions to its vicinity. These ions are drawn closer the greater the potential-difference between the electrode

and the solution. They can not, however, come in contact with an electrode without disruption of the water-shell on the surface of the electrode. If the potential-difference between the two electrodes is increased to equal the decomposition-potential of the electrolyte, the water-shells on the electrodes are disrupted and an electric current passes between the electrodes and the solution, being carried by ions that plate out on the electrodes, losing at the same time their charges and their water of hydration.

Two KCl solutions were prepared by accurate weighing, one 0.00106 per cent KCl and the other 0.00706 per cent KCl. Electrode vessel A was filled with 0.00106 per cent KCl and balanced in the bridge against a circuit consisting of a resistance R in series with a resistance r and the latter in parallel with a capacity C at 25°. The series resistance R represents that of the electrolyte and was determined at high frequency (1,500,000 cycles per second) and not changed during measurements at other frequencies. It was found

TABLE 2

f	C	
	Observed	Calculated
	μF	μF
500	1.460	1.490
1,000	1.342	1.332
1,500	1.253	1.241
2,000	1.199	1.189
2,500	1.160	1.150
3,000	1.124	1.113
3,500	1.093	1.082

that with change of frequency, a balance could not be obtained with C and R without the addition of a parallel resistance, r. Theoretically the capacity, C, is due to the ions that are attracted by the electrode but do not reach it, and the conductivity, $\frac{1}{r}$, is due to the ions which collide with the electrode.

Similar measurements were made with the 0.00706 per cent KCl solution.

If the capacity C (in microfarads, μF) is plotted against the square root of the frequency, $\sqrt{f}$, a curved line is obtained but if the logarithm of the capacity, *log C*, is plotted against the logarithm of the frequency, *log f*, a straight line is obtained. This is in harmony with theoretical considerations indicated by Warburg (1901), and measurements of Philippson (1921).

The graph of the 0.00106 per cent KCl solution may be expressed by the empirical equation

$$C = 1.303\, f^{-0.205}$$

The observed and calculated values are shown in table 1.

The graph of the 0.00706 per cent KCl solution may be expressed by the empirical equation

$$C = 4.085\, f^{-0.162}$$

Observed and calculated values are given in table 2.

Experiments with a colloid, agar. The Helmholtz double layer on the surface of the electrode acts as a capacity reactance. It is possible to imagine a Helmholtz double layer on the surface of a colloidal particle. If

TABLE 3

AGAR IN H_2O	CYCLES PER SECOND						WITH R SHORT CIRCUITED			
	1,500,000		3,000		1,000		f = 3,000		f = 1,000	
	Temperature	R	r	C	r	C	r	C	r	C
Electrode vessel A										
per cent	*degrees*			μF		μF	*ohms*	μF	*ohms*	μF
0.2	25.0	916.0	427	0.002	423	0.014	1,340	0.00023	1,339.0	0.00100
0.4	25.5	645.5	123.0	0.020	148.7	0.103	786	0.00050	788.8	0.00050
0.4	25.1	663.0	124.0	0.025	128.6	0.140	784	0.00056	786.6	0.00071
0.4	25.3	671	126.0	0.023	128.6	0.137	793	0.00060	794.5	0.00048
0.6	25.0	556	77.4	0.057	81.5	0.349	630	0.00082	632.4	0.00059
0.8	25.0	495	53.0	0.125	56.4	0.693	545	0.00100	547.3	0.00700
1.0	25.0	413	31	0.320	35.8	1.830	440	0.00045	441.4	0.01000
Electrode vessel B										
0.2	25.0	813	458	0.003	460	0.015	1,368	0.00028	1,370.0	0.00680
0.4	25.0	628.5	108	0.038	112.4	0.230	730	0.00071	733.5	0.00026
0.6	24.8	547	78.0	0.070	83.9	0.400	620	0.00110	622.7	0.00100
0.6	25.3	555	77.5	0.007	82.5	0.415	630	0.00107	632.3	0.00081
0.8	25.0	450	38.7	0.225	46.1	1.525	489	0.00063	486.4	0.01100
1.0	25.0	393	34.0	0.300	28.4	1.770	425	0.00200	428.4	0.01400

an electrode vessel is filled with a salt solution and its capacity C measured and then colloid added, the colloid particles on the above hypothesis should decrease the capacity C.

When agar is electro-dialyzed for a long time it cannot be peptized by heat and hence we were content with dialyzing agar against changing distilled water for 10 days and then drying it in an oven at 102°. Weighed amounts of agar were heated with weighed quantities of water in weighed flasks with reflux condensers until dissolved and then the water lost by evaporation restored (by weighing). The warm solutions were run into the electrode vessels and allowed to cool until they had set to form gels.

They were then connected in the bridge and measured. The results of agar dissolved in distilled water are shown in table 3.

It may be observed that the agar added electrolytes to the water and decreased its resistance.

A second series of experiments was made with dialyzed agar dissolved in 0.625 per cent KCl solution and shown in table 4.

It may be observed in table 4 that the agar added electrolytes to the salt solution and decreased its resistance. The effect of agar on capacity (C) was insignificant if any.

TABLE 4

AGAR IN 0.625 PER CENT KCl	CYCLES PER SECOND					WITH R SHORT CIRCUITED			
	1,500,000 R	3,000		1,000		f = 3,000		f = 1,000	
		r	C	r	C	r	C	r	C
Electrode vessel A									
per cent			μF		μF				
0	100	15	12.1			101.0	0.019	103.0	0.158
0.2	96	13	11.5			98.4	0.021	100.0	0.180
0.2	96	15	12.0			98.5	0.021	99.6	0.179
0.4	95.5	9.8	11.8			94.5	0.022	96.3	0.182
0.6	91.5					90.0	0.026	90.9	0.205
0.8	85					86.0	0.025	88.2	0.214
0.8	89	20	12.01			91.0	0.024	92.4	0.205
1.0	84					85.5	0.025	90.0	0.210
Electrode vessel B									
0	97	34.0	10.2	100	11.54	98.0	0.028	101.0	0.217
0.2	93	13	8.3	49.3	10.5	95.0	0.300	98.7	0.230
0.2	93	14	9.0	112	11.37	93.8	0.030	96.8	0.240
0.4	88	7.7	8.1	51.7	10.98	85.8	0.033	88.5	0.256
0.4	78	46	10.1	98.0	11.32	75.3	0.046	79.0	0.356
0.8	86	90	10.9	113.0	12.01	88.0	0.033	90.0	0.263
0.8	83	20	10.0	79.0	11.38	83.0	0.037	86.0	0.292
1.0	83	16.0	9.9	59.4	11.35	81.5	0.036	87.4	0.280

Polarization-capacity may exist at the surface of a living cell or other semipermeable membrane without the presence of a metallic conductor. It may be pictured in the following manner. To go back to the metal-electrodes, suppose a glass-tube containing an electrolytic solution is plugged with a metal-partition and a potential-difference set up between electrolyte 1 and electrolyte 2. The surface of the metal in contact with electrolyte 1 acts as one electrode and that in contact with electrolyte 2 acts as another electrode. Now suppose the thickness of the metal-par-

tition is decreased until it is only one atom thick. The same electric relations hold, that is to say, if electrode 1 is charged positively that side of the partition facing it will be negative and there will be a super-abundance of positive ions in its neighborhood. In the same way there will be a super-abundance of negative ions on the opposite side of the partition in the neighborhood of its surface. Since the partition is only one atom thick, its conductivity may be neglected, in which case it makes no difference what material it is made of. The essential characteristic of the partition is the water-shell on each side which acts as a film to keep out the ions; in other words, the partition acts essentially as a layer of bound water. The layer of positive ions on one side can not pass through to the layer of negative ions on the other side unless a sufficient potential-difference is created to disrupt the partition. We have here, then, a polarization-capacity without the necessity of a strictly metallic conductor. We might imagine the partition as made of a mono-molecular layer of fatty acid molecules or any other material that may be held in the form of a thin film between the surface of the cell and the metal and which is impermeable or poorly permeable to ions. If the voltage is sufficiently increased the partition is disrupted and the shell is destroyed but it is possible to imagine a certain number of ions passing through the partition without entirely abolishing the polarization. The partition is then the seat of a high resistance and a capacity-reactance.

Measurements on membranes on the surface of living cells. Each electrode is a platinized gold plate 15 mm. in diameter in a glass vessel filled with serum and fitted with a single bored rubber stopper. Defibrinated blood is introduced in a glass tube of 2 mm. bore closed at one end and centrifuged at 20,000 revolutions per minute in a vacuum until all the serum is pressed out from between the corpuscles. A short piece of the tube containing corpuscles (after being passed through the stoppers) is cut off at each end by a high speed steel disk and each end closed with a very thin piece of "cellophane" with the exclusion of air bubbles. Each stopper holding an end of the tube is then introduced into an electrode vessel. The cell constant of the tube is calculated afterwards from micrometer measurements of its length and bore.

The blood corpuscles in the electrode vessel are connected in the constant temperature box (kept at 25° ±0.1°) and balanced against a capacity C in parallel with a resistance r and in series with a resistance R. At any one frequency an infinite number of values of C, r and R may be found since either C, r or R may be changed within certain limits and the bridge brought into balance again by adjusting the other two but R may be varied only between O and the total impedance and is equal to the total impedance at infinite frequency. When, however, the frequency is increased to 1,500,000 the value of R cannot be changed appreciably as it is nearly equal to the

total impedance and hence R is first determined at 1,500,000 cycles and this value used in determining C and r at 1000 cycles.

Since in previous work it was found that the heat produced in centrifuging the corpuscles might be injurious, the centrifuge and motor were cooled to 0° before inserting the blood tubes and exhausting the air from the shield. In the following measurements on ox-corpuscles the time of centrifuging is noted in order to determine whether the time is adequate on the

TABLE 5

Ox-blood corpuscles (specific values)

NUMBER	TIME OF CENTRIFUGING	R	r	C
	minutes	*ohms*	*ohms*	*μF*
1	6	524	611	0.005,850
2	6	473	565	0.004,720
3	6	603	598	0.006,078
4	6	590	702	0.005,820
5	12	486	857	0.004,080
6	12	425	493	0.005,090
7	15	536	968	0.004,110
8	15	539	485	0.005,700
9	15	553	353	0.009,000
10	17	546	508	0.005,000
11	17	531	407	0.006,300
12	17	464	434	0.004,140
13	18	490	620	0.005,150
14	20	446	377.5	0.007,210
15	20	535	668	0.003,510
16	20	528	468	0.007,356
17	20	505	334	0.008,370
18	20	501	379	0.008,090
19	21	508	486	0.005,880
20	24	408	348	0.005,925
21	25	427	518	0.005,525
22	30	494	457	0.004,950
23	30	509	570	0.004,790
24	30	533	417	0.006,290
Average.....	17.6	507	526	0.005,788

one hand or too long on the other. The values are specific values and the specific resistance of the serum is 90 ohms.

Average specific impedance to low frequency currents = 1,033 ohms or about 12 times that of serum.

From table 5 it is evident that the duration of centrifuging may be varied from 6 to 30 minutes without affecting the result. Six minutes are sufficient for maximum resistance (r) and no injury resulting in lowered

resistance occurs in 30 minutes. As six minutes were required to exhaust the air no experiments of shorter duration were made.

Another series of measurements was made (table 6) at 3 frequencies: 1,500,000, 3000 and 1000. The values are not quite the same as those

TABLE 6

Ox-blood corpuscles (specific values)

$f = 1{,}500{,}000$ R	$f = 3{,}000$		$f = 1{,}000$	
	r	C	r	C
		μF		μF
756	293	0.00870	280	0.008700
640	139	0.02140	136	0.021400
702	293	0.01680	282	0.011000
595	228	0.01335	293	0.011750
560	239	0.01410	236	0.012800
670	366	0.00752	365	0.009940
872	165	0.03140	167	0.025200
954	282	0.02210	293	0.013900
1,066	450	0.01240	450	0.007190
940	330	0.01610	330	0.016950
960	515	0.00944	526	0.008240
918	323	0.01860	321	0.014600
848	559	0.01060	530	0.007800
Average...816	370	0.01557	324	0.012975

TABLE 7

Ox-blood corpuscles—electrode A

WASHED IN	CELL VOLUME	CYCLES PER SECOND					WITH R SHORT-CIRCUITED			
		$f = 1{,}500{,}000$	$f = 3{,}000$		$f = 1{,}000$		$f = 3{,}000$		$f = 1{,}000$	
		R	r	C	r	C	r*	C†	r*	C†
	per cent									
Serum	87	384	189	0.0220	189	0.152	558	0.00235	572	0.0165
0.93 per cent NaCl	95	424	301	0.0055	299	0.0084	718	0.00138	716	0.0099
1.2 per cent $CaCl_2$	96	404	271	0.0089	275	0.0634	667	0.00152	674	0.0010
8.8 per cent sucrose	93	573	190	0.0007	190	0.0032	1,946	0.000353	1,950	0.0065

* Spec. Res.

† Spec. Cap.

given in table 5. The reason for the difference is not clear and therefore we must admit of an uncertainty in the measurements and the true value may lie between those of table 5 and those of table 6.

Average specific impedance at low frequency = 1163 ohms or about 13 times as great as serum.

Further determinations were made using ox-blood corpuscles in electrode vessel A that had been used for agar. It was not possible to pack down the corpuscles at 20,000 r.p.m. for this large vessel and they contained 4 to 13 per cent of serum or washing fluid. Since this contamination might influence the results the corpuscles were washed with different isosmotic solutions to ascertain what effect this might have. The result are shown in table 7.

The variations in table 7 may be due to the presence in varying amounts of fluid between the corpuscles. In the last experiment isosmotic cane-sugar solution was used for washing and since its resistance is high it does not tend to short circuit the current which would otherwise pass through the corpuscles. Therefore, the measurements on corpuscles washed in sugar

TABLE 8

Hog-blood corpuscles

WASHED IN	CELL VOLUME	CYCLES PER SECOND					WITH R SHORT-CIRCUITED			
		R	f = 3,000		f = 1,000		f = 3,000		f = 1,000	
			r	C	r	C	r	C	r	C
	per cent						*ohms*	*μF*	*ohms*	*μF*
Serum	94	348	638	0.00260	638	0.0152	986	0.00069	986	0.00593
0.995 NaCl	96	363	624	0.00280	624	0.0138	974	0.00084	986	0.00566
1.37 per cent $CaCl_2$	98	392	921	0.00138	917	0.0062	1,305	0.00040	1,308	0.00304
9.5 per cent sucrose	95	392	3,944	0.00056	3,929	0.0007	4,323	0.000310	4,320	0.000314

solution should be comparable to those in tables 5 and 6. The change in C with frequency may be expressed by the equation

$$C = 219.3 \times f^{-1.325}$$

Since hog blood corpuscles precipitate more rapidly in the centrifuge than ox-corpuscles, measurements were made on them (table 8).

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ON THE RESPIRATORY CENTRE

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In 1923 Lumsden (1923 a) published the first of a series of papers in which he believed that he had proved the existence of four parts to the respiratory centre. It seemed, were his observations and explanations correct, and we had no reason to doubt them, that the depression or failure of one of these centres might explain the abnormal types of respiration observed with certain of the respiratory depressants, and that the site of attack of both respiratory stimulants and depressants might be more exactly located. These points led us to repeat the observations of Lumsden though we pursued almost from the first a slightly differing technique, the reasons for which will subsequently appear.

Lumsden in his first paper used the following technique. Recording tambours were strapped to thorax and abdomen. The vagi were freed, a blood pressure cannula put in one carotid and the other clamped. The parietal bone was trephined in front of the occipital ridge and a thin blunt chisel was inserted behind the tentorium and pushed rapidly to the base of the skull. The lower sections were made in a similar fashion (presumably through the cerebellum). Lumsden fails to give an exact anatomical description of the lesions produced by any of his sections. He gives no gross or histological definition of their levels, save in drawings, and no consecutive protocols of his experiments which would enable one to follow the course of his manipulation in detail in any experiment. This makes a repetition of his exact experiments difficult. In his paper he reports only on experiments performed on cats though he refers to other experiments performed on rabbits, dogs and monkeys. His findings are as follows. His first section "behind the corpora quadrigemina and above the pons causes no appreciable change in rate and depth." His second section, which might be anywhere between level one and a point about a millimetre or two above the striae acousticae (which lies approximately at the lower border of the cerebellar peduncles) caused at once the development of the

[1] The greater part of this work was carried out during tenure of the George Brown Memorial Fellowship by T. A. Sweet, who wishes also to express to the Trustees of the Banting Research Foundation his appreciation of the grant given for animal expenses.

inspiratory type of breathing "whether the fifth and tenth nerves had been cut or not." This inspiratory type which he subsequently terms "apneusis" consists in a sharp inspiration maintained for possibly some minutes. The inspiratory level may or may not be fully maintained and is succeeded by a deep expiration. Consequently he concludes that there is a higher controlling or pneumotoxic centre in the upper part of the pons. A section through the level of the striae leads to a convulsion followed only by gasping respiration. Section below the striae produces a gasping type of respiration with brief violent inspirations and occurs whether the vagi have been cut or not. Hence Lumsden concludes there is in lower medulla only a primitive respiratory centre from which gasping arises.

METHODS. Although an animal decerebrated below the tentorium and with carotids tied would probably suffer little pain on account of the lack of blood supply to the cerebrum, it seemed nevertheless advisable to us to decerebrate with a Sherrington guillotine or perform complete decortication prior to proceeding to the experiment proper. Further, as we were anxious to be sure that we could repeat our experiments as exactly as possible we felt it was necessary to have a more exact knowledge of the level of our lesions at the time that they were made. We removed the cerebellum and the Sherrington decerebration was in most of our experiments carried rather far forward so as to clear the anterior corpora quadrigemina and the rest of the occipital lobes removed with scalpel. Hemorrhage was then stopped. For such purposes we have found the employment of small pieces of tin, some 1.5 cm. long and 2 mm. wide folded in the middle and pressed together over a bleeding point, very convenient.

In our earlier experiments portions of the occipital and parietal bones were removed so as to uncover the central mass of the cerebellum. This was then split in the medial line and with a chisel-shaped knife the cerebellar peduncles were cut and the cerebellum scooped out. In our more recent experiments this technique has been greatly improved upon by making an incision with a circular saw through or close to the line joining the squamosal and parietal bones laterally, passing through the lamboidal ridge and across the rear of the occipital just above the occipital condyles and the foramen magnum. The bony tentorium was slit through with fine bone forceps and the whole mass of bone thus removed. This exposed the whole of the cerebellum and it was not difficult to raise the paraflocculus on one side and if necessary cutting through it approach the left cerebellar peduncles laterally, and cutting these through to cautiously roll back the cerebellum being careful to cut through the pial folds uniting it with the medulla and midbrain so as not to exert traction on other structures, until the right cerebellar peduncles came into view. These we then cut, and removed the whole cerebellum. Subsequently sections through the brain stem were made with a fine sharp eye knife and with a sawing up and down

movement so as to produce as little traction as possible on tissues as it was found that traction very readily upset respiration. This technique entails, of course, some loss of blood and some shock but in the successful experiments the ear reflexes returned promptly and the blood pressure was usually from 70-80 mm. of mercury. Respiration was usually regular and ample and varied in rate from faster to slower than normal.

As our original intention had been to study the effects of drug-stuffs we were anxious to get quantitative measurements of respiration and after trying various other methods we finally employed in most of our experiments, a body plethysmograph, which consisted of a galvanized iron cylinder flattened on one side into which slid a narrow board to which the animal was strapped. The cylinder had two outlets, one of which was taken to a Brodie's bellows recorder with a large capacity which we found convenient to cover with oiled silk. The other was used for taking temperature from time to time as, of course, temperature regulation was lost in most of our experiments, but the inclusion in the cylinder which lay on a hot water pan served to keep the temperature of the animal practically normal throughout the period of experimentation. The cylinder was made air tight at the upper end with plaster of paris which formed a wall at the junction of the shoulders and neck, thus not interfering with respiration and yet leaving the whole head and neck exposed. The brains were hardened in situ, removed and examined in gross and portions removed for histological study.

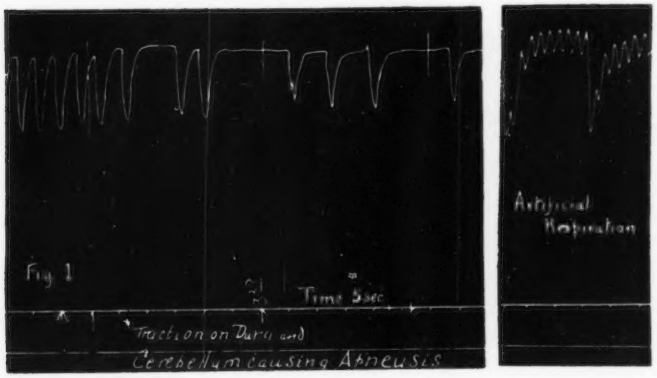
While we failed to confirm the statement made by Lumsden that a section through the brain stem between the corpora quadrigemina and the lower border of the cerebellar peduncles produced the inspiratory or apneustic type of breathing whether the vagus were cut or not, we can readily understand his mistake as in many cases we saw apneustic respiration lasting for shorter or longer periods after and indeed before such sections. These usually disappeared after a period of artificial respiration. (Fig. 1.)

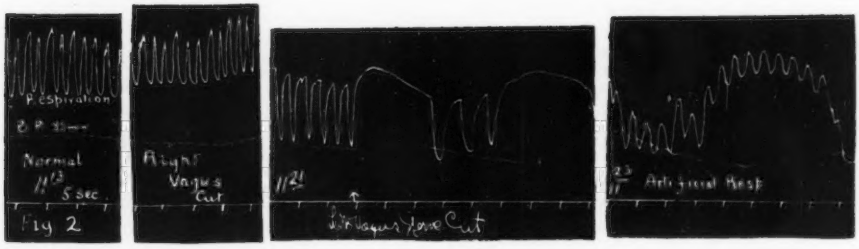
28. 3. Cat. 2.7 kgm. Decerebrated 3:21 p.m.
Respirations 19 per minute. Depth 14.
3. 23–3. 28. During removal of cerebellum considerable traction on pia which was followed by apneustic respiration irregular, 3–5 per min. persisted during artificial respiration periodicity 3–5 per min. 3:25 B.P. 150 mm. Apneustic during pump 13–14 per min. 3:29 Respiration natural but slow.

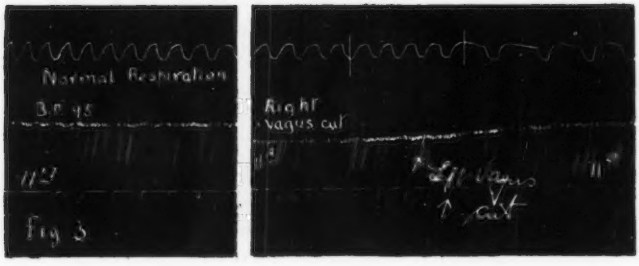
In successful experiments, however, we were able to make sections across the brain stem and frequently more than one at all levels between the lower border of the corpora quadrigemina down to a level 1 to 2 mm. below the striae acousticae without producing apneustic respirations if the vagi were intact. Cutting the vagi led to apneustic respiration. Of these experiments the following will serve as example. (Fig. 2.)

13. 2. 28. Cat 1.6 kgm.

9:45 a.m. Decerebrated, line of optic chiasma ventrally to middle thalamus dorsally. Occipital lobes removed, bleeding stopped. Cerebellum removed completely. Enclosed in plethysmograph. Initial rate of respiration 21–54, depth







18–24. During removal of cerebellum short period of apneusis. Rate settles down at 76 per min. volume 24 cc. minute vol. 1824, B.P. 85 mm. Hg.

10:48. Section, dorsally level mid-cerebellar peduncles on left, upper border of cerebellar peduncles on right. Ventrally almost reaches pontomedullary groove.

Complete save less than 1 mm. in places ventrally and a part of right brachium conjunctivum. Histologically only part of pyramidal tracts uncut ventrally and brachium conjunctivum on right side. Section produced some apneusis with shallow respiration; short period of artificial respiration. After removal of artificial at first slight apneustic quality.

11:12. Normal respiration. Rate 30 min. vol. 960, B.P. 92 mm.

11:13. Right vagus cut. Respiration slightly slower and shallower.

11:20. Rate 24. Vol. 864. B.P. 82 mm.

11:21. Left vagus cut. Apneusis lasting 5–20 seconds. Depth about 20. B.P. variable 50–95 mm.

11:22. Artificial respiration showing apneustic waves duration of about 25 sec. Then rate 45, depth approximately 50 cc. apneustic in quality.

11:25. Left vagus stimulated at 30 cm. the duration of apneusis was reduced from 45 sec. to 25 sec.

11:29. B.P. 50–75 mm. Skin stimulation at 10 cm. Some shortening of apneusis in some cases 35–20 8th nerves cut, no effect. Stimulation left 10th roots. Apneusis cut from 40 to 10–15 sec.

11:35. Section in midline to cut pyramidal decussation.

11:36. Artificial off, several gasps. B.P. 40 and after adrenalin larger more frequent gasps, rate 12 per min. one shallow apneusis.

We should at this point discuss the mechanism of apneustic respiration and try to explain the discrepancy between our results and those of Lumsden. First, it will be noted that our experiments agree with those of Trevan (1916) and Trevan and Boock (1922) who noted that sectioning the brain stem at various levels from roughly the upper third of pons to the middle of the superior corpora quadrigemina, produced a slowing and deepening of respiration but that after sectioning of the vagi the respiration showed a long drawn inspiratory phase doubtless corresponding to the apneustic respirations of Lumsden. Our experiments are in complete agreement with these authors. Further since these experiments were performed papers have appeared by Schoen (1929) and Teregulow (1929) who also have found that cutting the brain stem down to the striae does not produce apneustic respiration. Schoen used a technique which would not record apneustic respiration if it occurred and Teregulow does not describe the method by which his tracings were made. Neither of them explain Lumsden's findings. Secondly, as we found that in many cases apneustic respirations could be produced by trauma including hemorrhage into the section and hemorrhage under the brain stem, it seems quite probable that if Lumsden carried his sections through the cerebellum he would be very likely to produce pressure changes due to hemorrhage or shock such as would lead the respiration to become apneustic for long periods of time or even permanently. In experiments of this kind positive results are much more important than failures. Thirdly, sections made by either Lumsden or Trevan and Boock or ourselves, will inevitably cut through either the red nucleus or the rubro-spinal tracts. Now as was shown by

Magnus (1924) this entails the production of decerebrate rigidity and according to Magnus the section can be made anywhere above the striae acousticae. Magnus quotes certain experiments in which he attempted to cut the decussation of Forel or decussation of the rubro-spinal tracts in a cat. Some of our experiments with partial sections led us to be a little doubtful whether a stab wound such as was used by Magnus inserted into the midline immediately below the red nucleus would cut all the fibres leaving the nuclei. This led us to discuss the course of the rubro-spinal tracts with Prof. J. H. Craigie who advised us to write to Prof. J. W. Papez (1929) of Cornell University, who very kindly supplied us with a series of sectional drawings of this part of the brain stem from his book. According to Winkler and Potter (1914) the crossing of the rubro-spinal tracts takes place throughout the whole length of the red nuclei, and certainly slightly below it, whereas according to Papez the fibres ascend before crossing but from neither Winkler and Potter nor from Professor Papez could we obtain the location of the rubro-spinal tracts between the level of the lower border of the red nucleus and where they become defined as definite tracts at about the level of the mid-pons. Consequently, in order to ascertain whether it was cutting of the rubro-spinal tracts which, together with section of the vagus, led to the occurrence of apneustic respiration we made a section through the midline between the red nuclei. The results of such an experiment are indicated in the following protocol.

1. 2. 29. No. 70. Cat. 1.6 kgm. Decerebrated level optic chiasma and mid thalamus. Respiration recorded by tambour on chest.

10:15 Cut right vagus. 10:16 Cut left vagus. Artificial respiration during removal of cerebellum. Respiration regular 24 per minute. Longitudinal section in midline between optic thalami and corpora quadrigemina lower edge of section in upper pons. Microscopically complete section of rubro-spinal crossing. Prompt development of slow, shallow apneustic respirations 3 per minute. B. P. 68. Apneusis persisting in spite of prolonged artificial respiration.

The same result was obtained when the crossing was cut before the vagi when there was less danger of shock. We also, in other experiments, made two stab wounds laterally in the brain stem about opposite the middle of the cerebellar peduncles and carried these to the lateral margins of the pons, in this way cutting the rubro-spinal tracts, and after cutting the vagi apneustic respiration supervened. We would therefore attribute apneustic respiration to the general increase in tonus of the muscles of the chest and abdomen caused by cutting the rubro-spinal tract.

As the inclusion in a box made it impossible to observe the animal we tried other methods of recording respiration. The most successful was the insertion of a curved spoon-like extremity of a lever beneath the diaphragm, the longer end of the lever compressed a tambour which in turn was connected to a piston recorder. The tambour and lever were

connected to a frame which was attached to the upper end of the sternum and suspended so that movements of the animal did not interfere with the recording of the diaphragmatic movement. With this method apneustic respiration could be distinctly seen, but the information we were seeking, though indicated, was not definitely shown. We also tried recording with tambours from chest or abdomen. These, as Lumsden found, are difficult of interpretation but we succeeded in getting the information we required by simpler methods. When apneustic respiration had been produced opening the abdomen and placing a finger under the diaphragm enabled one to feel the course of the apneusis very distinctly, and also the state of the abdominal muscles at the same time. The following is a description of what was observed in this way in one case.

26. 1. 29. Cat 1.6 kgm. Decerebrated well forward. Cerebellum removed. Section transverse, upper border cerebellar peduncles left side and mid cerebellar peduncles right side to pontomedullary groove, very irregular, complete save less than 1 mm. of tissue left ventrally. Cut both vagi, apneustic respiration even with pump operating, period of 20 seconds: artificial respiration for 20 min. rigidity very marked in limbs and abdomen. On inserting finger from abdomen under diaphragm slow contraction of diaphragm. Diaphragm descending with increase in tonus of abdominal muscles. These relax sharply then slowly. Legs show increased tonus with diaphragm's descent.

In several cases in which cats were decerebrated so far forward that the red nuclei were not damaged, one or more sections were made through the central parts of the pons and medulla down to a level some 2 mm. below the striae acousticae and in these cutting the vagi did not lead to permanent apneustic respirations. In these cases postmortem examinations and histological sections showed that the rubro-spinal tracts had not been cut.

Expt. 12. 11. 1. 28. Cat 2 kgm. Decerebrated. Red nucleus area intact. 2:46 p.m. Cerebellum removed. 3:11 p.m. Resp. rate 78 min. vol. 1248, B.P. 49 mm. Hg. 3:12 Section level of upper third cerebellar peduncles dorso-ventrally to within less than 2 mm. of ponto medullary groove in mid-line extending laterally over about middle half the width of brain stem. (Rubro-spinal tract not cut.) Respiration somewhat irregular, rate from 76–44, min. vol. 1584–704, B.P. 103–80 mm. 3:16 Section about 2 mm. below the last and in a parallel plane. Just above (1 mm. or less) striae acousticae complete dorso-ventrally but not laterally. Area containing restiform body and rubro-spinal tract not cut. Respiration rate 45–57 min. vol. 900–2200 cc. finally regular. 3:20 3rd section about 1 mm. below the striae complete dorso-ventrally in mid-area but not laterally invading descending facial nucleus area but not rubro-spinal. Respiration rate 100–72 min. vol. 2000–1152 at times irregular. Vagi on both sides cut. Respiration 40, vol. 980. 3:22 4th section about 4 mm. above calamus complete dorso-ventrally in mid-area, inner edge nucleus ambigous cut but not rubro-spinal tract. Respiration rate 10–8 min. vol. 160–224. Artificial respiration for 3 min. 3:41 Rate 63, vol. 1260, B.P. 25 mm. After a section 2 mm. lower no voluntary respiration.

When apneustic respiration is produced by trauma or section and artificial respiration was given by a Hans Meyer pump producing alternate blast and exhaust we noted that the excursion of the bellow's writing lever with the pump was less than before the section and that frequently the respiratory tracing showed a series of large waves as in figure 1 which persisted often for long periods of time. We have no doubt that these are apneustic waves superimposed on the artificial respiration. The blood pressure frequently showed long waves of the same periodicity. Whether the expiration terminating the inspiratory apneusis was due to accumulation of carbon dioxide or was due to a central fatigue we do not know but we have some evidence that inhaling 7 per cent carbon dioxide decreased the length of apneusis.

We agree with Lumsden that apneusis can be inhibited by vagus stimulation though we have not been successful in finding the strength of current which rhythmically applied would convert apneustic respiration into an apparently normal one as shown in Lumsden's (1923 b) figure 1. However we found that stimulation of a cut vagus centrally with very weak currents often decreased the length of an apneutic period and in several cases when after cutting one vagus apneusis occurred we found that blowing into the trachea and distending the lung promptly cut short the apneustic inspiration.

We believe therefore that apneustic respiration is due to the tonus-producing activity of the medullary centres (which lie, and here we agree with Magnus, in the medulla below the striae acousticae) when these centres are unaffected by impulses from the red nuclei owing to cutting the rubro-spinal tract but that this tonus does not affect respiration deleteriously as long as the vagi are carrying sensory inhibitory impulses to the respiratory centre. The increased tonus of the respiratory muscles however leads to apneusis if these inhibitory impulses are not received. The apneustic centre of Lumsden thus disappears and with it his higher governing centre. He describes a lower gasping centre. We have frequently seen gasping respirations in our experiments and frequently alternating with normal respirations and superimposed on apneustic respirations. None of our sections nor those of Lumsden were lower than the supposed sites of the respiratory centre, i.e., in the comissural nucleus of the tractus solitarius (Ramon y Cajal, 1909), or substantia reticulosa at approximately the level of the calamus scriptorus (Bechterew, 1908). The appearance of gasps seems to have always occurred in cases of low blood pressure and we find no reason to believe that they are due to any new centre but solely to a radiation of more powerful impulses from the respiratory centre to anterior horn cells of other muscles than those normally employed in respiration.

Many experiments were carried out on cats, which were given ether, enclosed in the respiratory plethysmograph, the blood pressure recorded

and morphine given intravenously till respiration was markedly slowed. When adequate morphine had been given and the vagi were cut, the respiration became apneustic in type. (Fig. 3.) These apneustic-like respirations were always short in periodicity and in some cases inhalation of 7 per cent CO_2 in air increased rate, depth and total exchange.

SUMMARY

1. The respiratory centre is located in the lower part of the medulla.

2. Respiration is affected by the increase in tonus caused by the activity of the medullary tonus centres when the impulses from the red nucleus are cut off but not deleteriously as long as the vagus nerves are functional.

3. Cutting off of the impulses from the red nucleus and from the lungs by cutting the vagi leads to apneustic respiration.

4. Our work shows no evidence for more than one respiratory centre.

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THE INFLUENCE OF A MODERATE AMOUNT OF PHYSICAL TRAINING ON THE RESPIRATORY EXCHANGE AND BREATHING DURING PHYSICAL EXERCISE

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In another paper (Schneider, Clarke and Ring, 1927) we considered the effects of moderate physical training on the basal respiratory exchange, pulse frequency, and arterial blood pressure. We now desire to follow the changes in the position of the "crest-load," in the respiratory exchange, and in the minute-volume of breathing for two subjects while performing physical work, through a period of regular physical training and during an after-period when no exercise was taken, in order to determine how soon and to what extent moderate regular exercise may influence each. The training required the spending of approximately an hour every day for a period of two months in some form of vigorous physical exercise. In the early fall this consisted mostly of tennis and later on of handball and swimming, with occasional running on an indoor track.

One of our subjects, G. C. R., was a young man 5 feet 10½ inches tall and weighed 140 pounds. He had spent the summer prior to the experiments at the sea-shore and had taken part in many outdoor sports, especially swimming; so that when he left there on August 15, he was in excellent physical condition. From then until the time our experiments began, he did almost no physical work.

E. C. S. was of almost the same build as G. C. R., being 5 feet 9½ inches tall and weighing 135 pounds. He was a man of middle age who usually enjoyed good health, but during the year of this study he had an attack of appendicitis. He had led a sedentary life for three months before our experiments began and was quite unfit.

Once a week during an afternoon each man was examined while at work on a bicycle ergometer of the Krogh type. After a period of rest, the subject mounted the bicycle, put the mouth-piece of a respiratory apparatus in place, and then sat as quietly as possible for a preliminary period of three minutes to allow the body to get into equilibrium; following this the expired air was collected in a Douglas bag for 10 minutes. Mueller inspiratory and expiratory water-seal-valves were employed. The subject was then

put to work on the bicycle ergometer. The first load of work was usually 2000 ft. lbs. per minute. After three minutes of preliminary work, to allow the body to get into equilibrium, the expired air was collected for two minutes. The subject then rested for from 10 to 20 minutes, after which he did 4000 ft. lbs. of work according to the plan employed for the first work period. Additional loads were carried in turn until an over-load was reached.

THE CREST-LOAD. The percentage of carbon dioxide in the expired air of a work period has been employed by Briggs (1920) to determine the amount of work that constitutes a crest-load. So long as the percentage of the exhaled carbon dioxide rises proportionately with added increments of work the load is considered normal. According to Briggs the crest-load is reached when the oxygen supplying mechanisms, working at full capacity, just meet the oxygen need of the reconversion process of the muscles. With this load the percentage of exhaled carbon dioxide is at its maximum. A further increase in the load of work becomes an overload and is associated with the escape of lactic acid from the muscles into the blood, and a proportionate reduction in the formation of carbon dioxide. When this occurs the percent of carbon dioxide in the exhaled air is less than it is with the crest-load.

The crest-load should be raised by physical training and lowered by disease. Just how much it can be shifted upward by training, and at what rate, has not heretofore been determined. Our experiments show that it is shifted upward by moderate daily exercise and downward by the neglect of physical exercise. We have plotted the curves for the percentage output of carbon dioxide in figures 1 and 2 to show the position of the crest-load for our two subjects during the entire period of study of the effects of training and non-training. The first curve for each subject is typical for his pre-training period. Regular physical exercise was begun October 8 and 12 respectively by E. C. S. and G. C. R.

The crest-load of E. C. S. (see fig. 1) prior to training was very low, only 2000 ft. lbs. This was advanced to 4000 ft. lbs. with his first week of training and to 6000 ft. lbs. by the end of the second week, where it remained during the next two weeks. Later there occurred a further advance to 7000 ft. lbs., which was reached at the end of the fifth week of training. The crest-load, with the exception of one determination, remained at 7000 ft. lbs. for the following four weeks, after which time it shifted back to 6000 ft. lbs. After the Thanksgiving recess E. C. S. neglected his exercise and more and more gave only a few minutes a day to setting up exercises. All exercise was omitted after December 12. Early in January this subject underwent an appendectomy and was not again tested for the position of his crest-load until February 22. At that date the crest-load was not well defined, but was not clearly above 2000 ft. lbs. Thereafter it ranged between 4000 and 5000 ft. lbs.

G. C. R. (see fig. 2), who was in better condition than E. C. S., showed a crest-load of 6000 ft. lbs. in the pre-training period. His crest-load advanced to 7000 ft. lbs. during the first week of training, then more

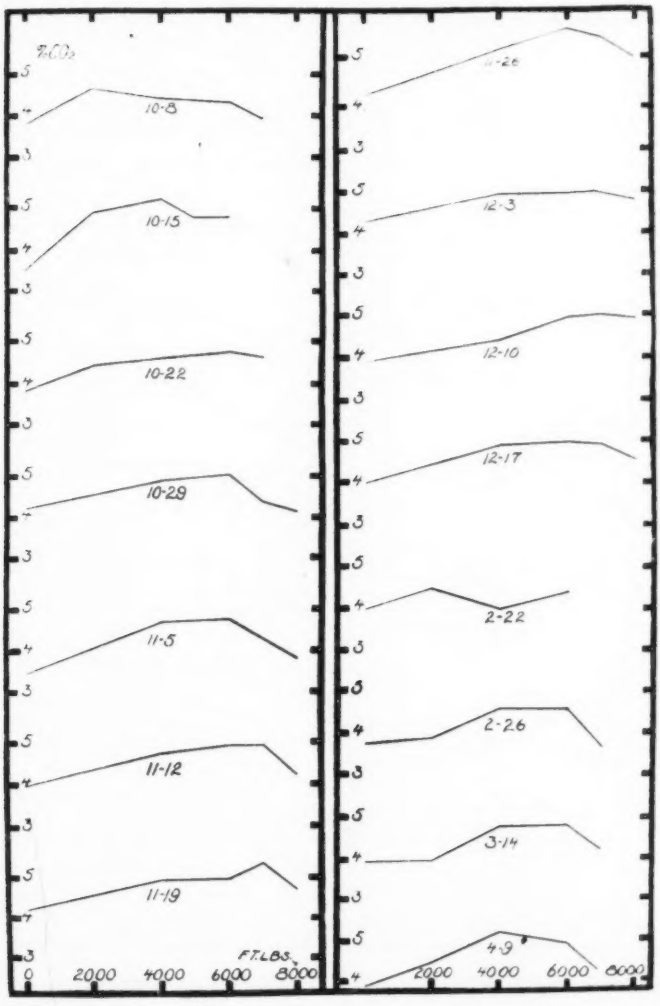

Fig. 1. Curves for E. C. S. showing the percentage output of CO_2 and position of the crest-load with increasing loads of work. These are given at intervals of a week during and after the period of training. Training was discontinued after December 12 (12–10). The ordinates give the per cent of CO_2; the abscissae, the load of work.

slowly to 8000 ft. lbs. by the end of the fifth week, and finally to 9000 ft. lbs. at the end of the seventh week of training. With the easing off of

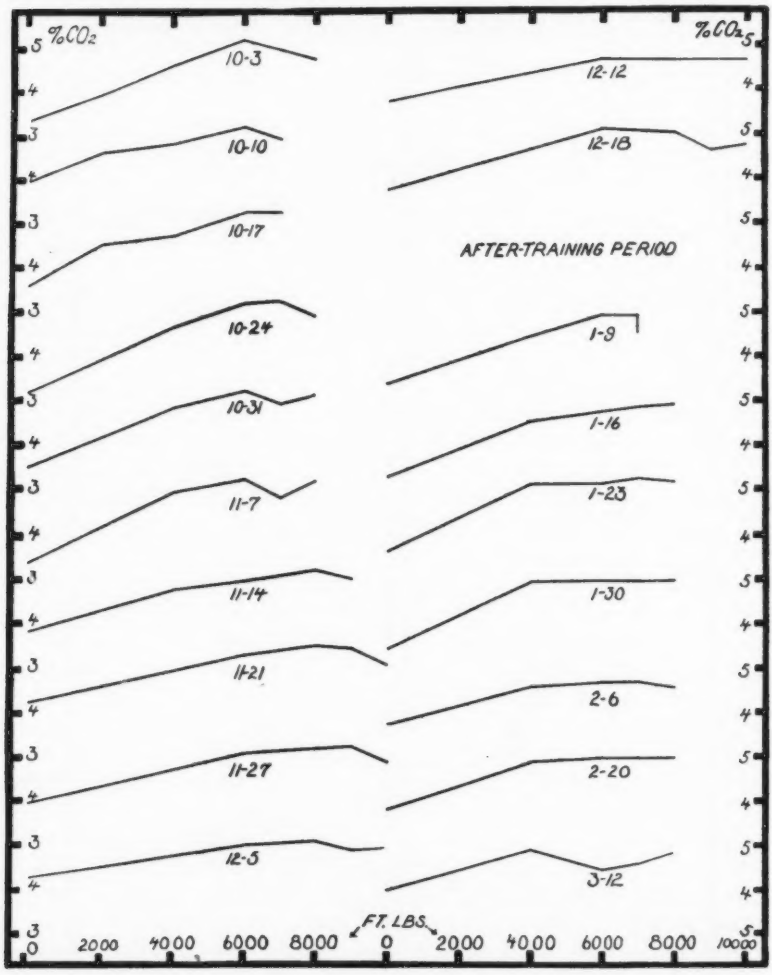

Fig. 2. Curves for G. C. R. showing the percentage output of CO_2 and position of the crest-load at intervals of a week during and after training.

training at the Thanksgiving recess, it quickly dropped back to 8000 ft. lbs. The crest-load could not be so clearly defined after the period of train-

ing as during the period of activity. The slump in the crest-load began as training tapered off, but the crest-load was still nearly 8000 ft. lbs. for five weeks of the post-training period. Later, when it could not be definitely placed, the carbon dioxide percentage in the expired air was practically the same for loads of 4000, 6000, and sometimes 8000 ft. lbs. The crest-load was then evidently not greater than 6000 ft. lbs.

The above results show that even the first week of moderate physical training increases the load-carrying ability, but that from 5 to 7 weeks of faithful training are required to bring about the full effect. They also show that any let-down in the amount of daily work at once reduces the

TABLE 1

E. C. S.—4000 foot pounds

DATE	CO_2	O_2	CO_2	O_2	R.Q.	MINUTE VOLUME	RESPIRATION FREQUENCY
Training period							
	per cent	*per cent*	*liters*	*liters*		*liters*	
10/ 8	4.53	3.37	1.78	1.33	1.339	39.32	20
10/15	5.30	4.86	1.80	1.65	1.091	34.00	15
10/22	4.66	3.72	1.83	1.46	1.253	39.30	19
10/29	4.97	4.45	1.56	1.43	1.092	32.18	18
11/ 5	4.78	4.16	1.68	1.47	1.149	35.22	20½
11/12	4.81	4.27	1.60	1.42	1.126	33.36	19½
11/19	5.04	4.57	1.58	1.44	1.103	31.40	18
11/26	5.26	5.02	1.44	1.37	1.048	27.33	14½
12/ 3	5.00	4.70	1.60	1.57	1.016	31.98	14
12/10	4.46	4.35	1.33	1.29	1.025	29.79	17½
After period							
2/22	4.19	3.72	1.55	1.38	1.126	37.00	20
2/26	4.66	4.32	1.58	1.46	1.079	33.84	20
3/14	4.86	4.57	1.55	1.46	1.064	32.00	19
4/ 9	5.28	5.10	1.57	1.52	1.053	29.78	16

crest-load, but that even with complete neglect of exercise some degree of the beneficial gain is maintained for several months.

EFFECTS OF TRAINING UPON VARIOUS FACTORS WITH A GIVEN LOAD OF WORK. In tables 1, 2, and 3 are given the data for loads of 4000, 6000, and 7000 ft. lbs. for E. C. S. during and after the period of training; and in tables 4 and 5 similar data are given for G. C. R., when he carried loads of 6000 and 8090 ft. lbs.

The percentage changes of CO_2 and O_2 in expired air. Others (Bainbridge and Anrep, 1923) have pointed out that for the same load of work the percentage of carbon dioxide in the expired air is roughly 6 for the trained man

TABLE 2

E. C. S.—6000 foot pounds

DATE	CO_2	O_2	CO_2	O_2	R.Q.	MINUTE VOLUME	RESPIRATION FREQUENCY
			Training period				
	per cent	*per cent*	*liters*	*liters*		*liters*	
10/ 8	4.46	3.37	2.358	1.782	1.323	52.86	
10/15	4.84	3.98	2.363	1.943	1.216	48.82	21
10/22	4.84	3.85	2.032	1.617	1.257	42.00	17.5
10/29	5.12	4.43	2.130	1.675	1.156	41.61	19.5
11/ 5	4.82	4.30	2.225	1.985	1.121	46.16	22
11/12	5.08	4.89	2.158	2.077	1.039	42.47	20
11/19	5.19	4.82	1.982	1.840	1.077	38.18	20
11/26	5.74	5.47	1.999	1.905	1.049	34.82	17.5
12/ 3	5.04	4.79	2.054	1.952	1.052	40.76	21
12/10	5.17	4.88	1.969	1.858	1.060	38.08	18
			After period				
2/22	4.48		2.240			50.00	23
2/26	4.69	3.88	2.348	1.942	1.209	50.06	22
3/14	4.88	4.09	2.330	1.953	1.193	47.76	20
4/ 9	5.00	4.41	2.186	1.928	1.134	43.71	20

TABLE 3

E. C. S.—7000 foot pounds

DATE	CO_2	O_2	CO_2	O_2	R.Q.	MINUTE VOLUME	RESPIRATION FREQUENCY
			Training period				
	per cent	*per cent*	*liters*	*liters*		*liters*	
10/ 8	4.07	3.03	2.40	1.79	1.343	59.04	
10/15	4.86	4.27	2.41	2.12	1.138	49.64	19
10/22	4.74	3.67	2.15	1.67	1.291	45.40	19
10/29	4.43	3.96	2.15	1.92	1.119	48.60	23
11/12	5.05	4.78	2.36	2.23	1.056	46.69	21
11/19	5.40	5.07	2.28	2.14	1.065	42.20	20
11/26	5.57	5.37	2.09	2.02	1.037	37.57	18
12/ 3	5.11	5.14	2.16	2.18	0.994	42.33	19
12/10	5.35	5.11	2.35	2.24	1.047	43.82	20
			After period				
2/26	3.80	3.07	2.51	2.03	1.238	66.10	34
3/14	4.36	3.66	2.46	2.06	1.191	56.40	25
4/ 9	4.33	3.82			1.133	54.14	23

as against 4 for the untrained man. It is also known that the trained man absorbs a larger percentage of oxygen from the inhaled air. Both of our subjects showed these changes, but G. C. R. in lesser degree than E. C. S. The extent of the changes was greatest when heavy loads were carried. Thus when E. C. S. carried a load of 4000 ft. lbs., the average percentage of carbon dioxide in the exhaled air was 4.86 during the early training, 4.94 during the late training, and 4.75 in the after training period; with a load of 6000 ft. lbs., it was 4.81 during the early, 5.29 during the late, and 4.76 in the after training period; and with a load of 7000 ft. lbs., it was 4.52 during the early, 5.36 during the late, and 4.16 in the after training period.

The percentage of oxygen absorbed also showed an increase with training. The average percentage with a load of 4000 ft. lbs. was 4.10 during early training, 4.66 during late training, and 4.43 after training; with a load of 6000 ft. lbs., it was 3.91 during early training, 4.99 during late training, and 4.13 after training; and with a load of 7000 ft. lbs., it was 3.73 during early training, 5.17 during late training, and 3.52 after training.

It is evident that when the load carried was an over-load the percentage of oxygen absorbed was less than with a normal-load. Thus at first, when out of training, loads of 6000 and 7000 ft. lbs. were over-loads for E. C. S. When out of training with a load of 4000 ft. lbs., his percentage of oxygen absorption was more than 4; while with loads of 6000 and 7000 ft. lbs., it averaged 3.91 and 3.73 respectively. During training, when the last two loads had come to be normal and crest-loads, the percentage of oxygen absorption was raised to 4.99 and 5.17 respectively.

The time required to develop these percentage changes in the gases of the expired air was in the neighborhood of from 4 to 5 weeks. Both E. C. S. and G. C. R. trained conscientiously up to November 28. It will be observed in tables 1, 2, 3, 4, and 5 that the percentage exhalation of carbon dioxide and absorption of oxygen was greatest for the weeks just preceding and after that date. Both men show a slump early in the after training period.

Minute-volume of breathing. It is well known that the trained man breathes much less air for the same accomplishment than does the untrained subject. Both of our subjects demonstrated this response with moderate training—see tables 1, 2, 3, 4, and 5. By averaging the data for the early training, late training and post-training periods, it will be found that E. C. S. breathed, with a load of 4000 ft. lbs., 36.2, 30.1, and 33.2 liters respectively; with 6000 ft. lbs., 46.3, 35.5, and 47.9 liters respectively; and with 7000 ft. lbs, 50.7, 41.5, and 58.9 liters respectively. G. C. R., who was in better condition, breathed, for the same three periods, with a load of 6000 ft. lbs., 39.2, 34.6, and 39.4 liters respectively; and with 8000 ft. lbs., 56.5, 46.9, and 52.8 liters respectively.

The effect of physical training on the minute volume of breathing during

effort is slightly manifest within the first or second week, but the maximum decrease is not attained for from 4 to 6 weeks. Our data do not show how soon after training this effect passes off, but it is evident that the influence has mostly disappeared within from 4 to 6 weeks.

The minute-volume exchange of carbon dioxide and oxygen and the respiratory quotient. It is well known (Bainbridge and Anrep, 1923) that the trained man can perform a given amount of work with a smaller consumption of oxygen and a smaller output of carbon dioxide than the untrained

TABLE 4

G. C. R.—6000 foot pounds

DATE	CO_2	O_2	CO_2	O_2	R.Q.	MINUTE VOLUME	RESPIRATION FREQUENCY
			Training period				
	per cent	*per cent*	*liters*	*liters*		*liters*	
10/ 3	5.21	5.41	1.97	2.04	0.963	37.74	
10/10	5.29	4.39	2.05	1.70	1.210	38.81	22
10/17	5.35	5.26	2.08	2.04	1.017	38.90	21
10/24	5.27	5.14	2.18	2.13	1.025	41.34	22
10/31	5.31	5.20	2.26	2.21	1.021	42.48	26
11/ 7	5.19	5.11	2.05	2.02	1.016	39.43	26
11/14	5.04	5.26	1.96	2.05	0.958	38.98	24
11/21	5.36	5.54	1.90	1.97	0.994	25.50	15.5
11/27	5.16	5.14	1.91	1.91	1.004	37.06	25
12/ 5	5.04	5.04	1.86	1.86	1.000	36.93	21
12/12	4.77	4.95	1.85	1.92	0.964	38.86	23.5
12/18	5.20	5.29	1.88	1.91	0.888	36.14	17
			After period				
1/ 9	4.98	5.04	1.86	1.88	0.988	37.29	22
1/16	4.80	4.98	1.86	1.93	0.964	38.70	24
1/23	5.16	5.29	2.09	2.15	0.975	40.61	28
1/30	5.09	5.25	1.87	1.94	0.969	36.86	25
2/ 6	4.92	4.92	2.08	2.08	1.000	42.37	28
2/20	4.83	4.83	1.96	1.96	1.000	40.57	28
3/12	5.15	5.02	1.94	1.89	1.026	37.65	24.5

man. The amount of exercise taken by our subjects resulted, in the course of time, in a small decrease in the output of carbon dioxide; while in the usage of oxygen E. C. S. showed an increase, and G. C. R. showed no change with loads of 4000, 6000, and 7000 ft. lbs. and only a moderate decrease with loads of 8000 ft. lbs. and more.

The difference in the output of carbon dioxide is best seen when the data are averaged. We have averaged the early, late, and post training data and find that for E. C. S. the volume of carbon dioxide exhaled per minute

was, with a load of 4000 ft. lbs., 1.74, 1.49, and 1.56 liters respectively; with 6000 ft. lbs., 2.22, 2.00, and 2.28 liters respectively; and with 7000 ft. lbs., 2.28, 2.22, and 2.44 liters respectively. G. C. R. eliminated, with a load of 6000 ft. lbs., 2.07, 1.91, and 1.96 liters respectively; and with 8000 ft. lbs., 2.86, 2.40, and 2.55 liters respectively.

The amount of oxygen used by E. C. S. was practically the same both when in and out of training for a load of 4000 ft. lbs; but, with a load of 6000 ft. lbs., the consumption of oxygen per minute rose from an early

TABLE 5

G. C. R.—8000 foot pounds

DATE	CO_2	O_2	CO_2	O_2	R.Q.	MINUTE VOLUME	RESPIRATION FREQUENCY
Training period							
	per cent	*per cent*	*liters*	*liters*		*liters*	
10/ 3	4.81	4.20	3.01	2.62	1.145	62.50	
10/24	5.04	4.65	3.23	2.99	1.084	64.36	32
10/31	5.21	5.13	2.56	2.52	1.016	49.11	28
11/ 7	5.26	5.24	2.64	2.63	1.004	50.13	26
11/14	5.29	5.43	2.52	2.58	0.974	47.58	26
11/21	5.57	5.92	2.10	2.23	0.941	37.66	19
11/27	5.22	5.33	2.44	2.49	0.979	46.51	27
12/ 5	5.13	5.09	2.54	2.52	1.008	50.73	29
12/12	4.76	4.76	2.51	2.51	1.000	52.65	32
12/18	5.10	4.83	2.56	2.42	1.056	50.20	31
After period							
1/16	4.95	4.86	2.60	2.55	1.019	52.55	34
1/23	5.15	5.29	2.34	2.40	0.974	45.36	28
1/30	5.14	5.38	2.47	2.59	0.955	48.11	31
2/ 6	4.87	4.77	2.60	2.54	1.021	53.35	34
2/20	4.87	4.86	2.54	2.54	1.002	52.19	32
3/12	4.88	4.73	2.59	2.51	1.031	53.08	36
4/ 7	5.08	4.85	2.48	2.37	1.048	48.87	37
4/11	4.54	4.39	2.59	2.50	1.034	57.01	40

training average of 1.76 liters to 1.95 liters in the late training period; with a load of 7000 ft. lbs., it rose from 1.88 in the early training period to 2.15 liters in the late training period. This increased usage of oxygen for the heavier loads is probably accounted for by an improvement in the circulation of the blood. At first both loads, 6000 and 7000 ft. lbs., were distinctly over-loads; while later they came to be a normal-load and crest-load respectively. If the reconversion of lactic acid into its precursor, glycogen, becomes more adequate as a result of training, then an increased usage of oxygen is to be expected.

For G. C. R. a load of 6000 ft. lbs. was at first his crest-load, but during the period of training it became a normal load. It is interesting to find that, like E. C. S., for a load of 4000 ft. lbs. the amount of oxygen consumed was unaffected by training. With a load of 8000 ft. lbs. G. C. R. reduced his oxygen consumption by training. His average usage per minute for this load was 2.69 liters during early training, and 2.44 liters during late training.

The interpretation of these changes in the consumption of oxygen and the output of carbon dioxide is best made in terms of the respiratory quotient. Our respiratory quotients are in no sense indicative of the kind of metabolism. Most of them are above unity—see tables 1, 2, 3, 4, and 5—and therefore indicate that lactic acid is being thrown into the blood during the period of work. The conspicuous feature of all our data is the fact that the respiratory quotient was decreased by training. The average respiratory quotients for E. C. S. in the early, late, and post training periods were, with a load of 2000 ft. lbs., 1.194, 1.048, and 1.076 respectively; with 6000 ft. lbs., 1.238, 1.059, and 1.177 respectively; and with 7000 ft. lbs., 1.223, 1.036, and 1.187 respectively. The averages for G. C. R. for the same periods were, with a load of 6000 ft. lbs., 1.054, 0.990, and 0.999 respectively; and with 8000 ft. lbs., 1.062, 0.982, and 1.029 respectively.

The decrease in the respiratory quotient indicates that one or the other, or both, of two things happened as the result of training. Either the reconversion of lactic acid into the precursor, glycogen, was more adequate during work; or the muscles were better buffered so that less lactic acid escaped from them during the period of training than when they were out of training.

The time required for training to affect the intake of oxygen, the output of carbon dioxide, and the respiratory quotient can be determined from tables 1 to 5. For G. C. R. there was a definite decrease in the respiratory quotient with a load of 6000 ft. lbs. in five weeks and with 8000 ft. lbs. in three weeks. E. C. S. showed some change during the first two weeks, but this was more definite and permanent after the fourth or fifth week. This gain was gradually lost in the post-training period, but had not wholly disappeared in four months.

SITTING AT REST ON THE BICYCLE ERGOMETER. It should be recalled that as a preliminary to the work on the ergometer the subject sat at rest, and that during a portion of this time his expired air was collected. The data for the rest observations on G. C. R. are given in table 6. It will be noted that the percentage output of carbon dioxide and intake of oxygen from respired air show a steady increase up to the end of November; and that, in the post-training period, they again practically return to the pre-training level. E. C. S. showed a similar response. Thus in the early training,

late training, and post-training periods the average percentage output of carbon dioxide was 3.90, 4.22, and 3.95 respectively; and the percentage intake of oxygen, 4.52, 5.17, and 4.39 respectively.

The minute volume of breathing during rest showed no distinct change either during or after training in either subject.

The minute-volume output of carbon dioxide and consumption of oxygen show a steady increase up to the end of the time that training was faithfully practiced, and thereafter on into the post-training period they decrease. The data for G. C. R., that bear out the above statement,

TABLE 6

G. C. R.—rest, sitting on bicycle

DATE	CO_2	O_2	CO_2	O_2	R.Q.	MINUTE VOLUME	RESPIRATION FREQUENCY
	Training period						
	per cent	*per cent*	*liters*	*liters*		*liters*	
10/ 3	3.45	4.35	0.236	0.298	0.793	6.84	
10/17	3.68	4.35	0.251	0.297	0.846	6.83	16
10/24	3.29	4.37	0.235	0.313	0.753	7.16	14.5
10/31	3.57	4.28	0.251	0.302	0.834	7.05	17.5
11/ 7	3.44	4.10	0.258	0.307	0.839	7.49	16
11/14	3.88	4.92	0.245	0.311	0.788	6.31	10
11/21	4.30	5.22	0.258	0.314	0.814	6.00	8
11/27	4.04	4.77	0.266	0.314	0.847	6.59	9
12/ 5	4.34	5.37	0.257	0.319	0.808	5.94	6.5
12/12	3.81	4.20	0.279	0.308	0.907	7.33	7
12/18	3.86	3.91	0.287	0.290	0.987	7.44	6
	After period						
1/ 9	3.43	3.58	0.312	0.326	0.958	9.11	12
1/23	3.69	4.60	0.220	0.275	0.802	5.97	7
1/30	3.45	3.74	0.265	0.287	0.922	7.68	7
2/ 6	3.75	4.08	0.313	0.345	0.909	8.44	8.5
2/20	3.65	4.23	0.221	0.257	0.863	6.06	14
3/12	4.01	4.16	0.274	0.284	0.964	6.83	13

are given in table 6. E. C. S. had an average output of carbon dioxide in the early, late, and post-training periods of 210, 232, and 249 cc. respectively and an intake of oxygen of 241, 283, and 291 cc. respectively. Just why the post-training quantities remain high for E. C. S. is not evident from data at hand. It should be recalled, however, that he was ill and underwent an appendectomy early in January.

The resting metabolism, calculated for a square meter of body surface, was definitely increased for both of our subjects by physical training. It was raised in G. C. R. from an average of 50.7 calories in the early training

period to 52.8 calories in the late training period, and in E. C. S. from 40.3 calories in the first to 46.9 calories in the second period.

It seems impossible to harmonize these data on the resting metabolism with our observations on the basal metabolism which were published some time ago (Schneider, Clarke, and Ring, 1927). The data there given showed G. C. R. to have an average basal metabolism per square meter of body surface per hour of 39.3 calories when out of training and 38.7 calories when in training, and E. C. S. to have an average of 36.8 calories when out of training and only 34.3 calories when in training. The observations on basal metabolism dealt with the same period of training as that from which the data of this paper were obtained.

SUMMARY

Moderate physical training, about an hour daily, increased the load-carrying ability within one week but the full effect is only observed after 5 to 7 weeks. Any let down in the regularity or amount of daily work soon reduces the load-carrying ability, but even with complete neglect of exercise some of the gain in power is maintained for several months.

The percentage of carbon dioxide exhaled and of oxygen absorbed during work is increased by training, the full effects are reached within 4 or 5 weeks. These soon drop back to the pre-training level when training is discontinued.

The minute-volume of breathing for any given load of work decreases with training, and reaches its lowest level in from 4 to 6 weeks. This effect disappears within 4 to 6 weeks after training is discontinued.

In one subject the absorption of oxygen was increased as training changed overloads to normal or crest-loads. In another subject a slight decrease in the oxygen consumption of work was evidenced as training proceeded. A decrease in the respiratory quotient of work during training suggests a gain in ability to more adequately reconvert lactic acid into its precursor.

The effects of physical training are manifest, in the subject sitting at ease, by a higher percentage output of carbon dioxide and intake of oxygen and by a higher metabolism per square meter of body surface.

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THE RELATIONSHIP OF CHANGES IN BLOOD-FLOW THROUGH AN EXTREMITY TO (1) CHANGES IN TEMPERATURE OF TISSUES, (2) DIFFERENCES IN OXYGEN CONTENT OF THE ARTERIAL AND VENOUS BLOOD, AND (3) CARDIAC OUTPUT

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In 1909, Hewlett and Van Zwaluwenburg studied the volume flow of blood in the arm by the use of a plethysmograph. They state, "We believe that the method here described determines the rate of peripheral blood flow in the arm with rough accuracy, the error in favorable cases not exceeding twenty per cent." In 1911, Stewart described a calorimetric method which he considered more accurate for determining the blood flow. Taylor (1922) (1923) preferred the calorimetric to the plethysmographic method and modified Stewart's apparatus so that determinations could be carried out in the horizontal position. Recent work by Sheard (1926), by Kegerreis (1926), by Brown (1926), and by Harris and Marvin (1927) has led them to doubt the value of calorimetric studies for determining the blood flow through extremities. Sheard states, "Calorimetric methods and data cannot be used to determine the quantity or rate of blood flow." Harris and Marvin demonstrated the inaccuracy of Stewart's calorimetric method which assumed that the temperature of the blood returning from the part immersed was the same as that of the calorimeter fluid. A thermocouple was inserted into one of the veins of the hand, which was immersed in water and it was found that the temperature of the venous blood was different from the temperature of the fluid in which the hand had been placed for ten minutes.

The unsatisfactory results which have been obtained from the use of the plethysmographic and calorimetric methods for determining the volume flow of blood in the extremities of man have led to the study of other methods. The purpose of this paper is to report these studies, which include determinations of the oxygen-content of the blood from the femoral artery and vein, determinations of the temperature of the tissues in various parts of the leg, and measurements of the amount of blood which flows from the femoral vein in a given unit of time.

In addition to the idea that such studies as are here reported might lead

to a useful method in examining patients with diseases of the circulation of the extremities, it was thought that they might throw some light upon the argument which existed between Heidenhain and Gaskell (1878) concerning the relationship between changes in temperature and alterations in blood flow. Heidenhain assumed in his work that a rise in temperature indicated a dilatation of blood vessels and that a fall in temperature always denoted a constriction. Gaskell reported the effects of nerve section on the blood flow and temperature changes in skeletal muscle. When the blood flow had reached its maximum, the temperature had not begun to rise. When the temperature reached its maximum, the rate of blood flow had returned to the normal, although the temperature did not return to the height it possessed before the section until fifteen minutes afterward. Gaskell concludes by saying that the temperature at any moment is not an indication of the state of the blood vessels at that moment, but that, if a rise of temperature in the muscle is really due to an increase of blood flow through the muscle, then the heating effect of the greater supply of blood takes a long time to attain its maximum, and a still longer time to disappear.

METHOD. All of the experiments were performed upon dogs. Morphine, chloralose, or barbital were used as anesthetics. The various types of anesthetics which were employed did not give different results. Six experiments were performed. Approximately two hours before the experiment was to be begun, the animal was given a sufficient amount of either morphine or chloralose or barbital for anesthesia. In all instances 30 milligrams, per kilogram of body weight, of heparin was given as an anticoagulant. The femoral vessels were then exposed on one side and a T-shaped cannula was placed in the femoral vein. A rubber tube was placed on the side arm of the cannula. This rubber tube was clamped except during the time that specimens were being collected. This allowed the blood to flow uninterruptedly from the leg through the cannula and to the heart. In four experiments frequent observation of the following factors were made: 1, pulse rate; 2, rectal temperature; 3, room temperature; 4, temperature of subcutaneous tissues of foot; 5, temperature of subcutaneous tissues of lower leg; 6, temperature of muscle of thigh; 7, oxygen content of blood from the femoral artery and the femoral vein and the right side of the heart; 8, oxygen consumption of the animal, and 9, outflow of blood from the femoral vein per unit of time. In two experiments, the oxygen content of the blood from the right side of the heart and the total oxygen consumption were not determined.

Temperature determination. A thermocouple as used by Brooks and Jostes (1924) was employed in the temperature determinations on the extremity. The needle was inserted successively into the foot, the subcutaneous tissues of the lower leg, and the muscle of the thigh. The

readings were obtained a few seconds before blood samples were collected from the femoral vein.

Total flow through the femoral vein. The amount of blood which returned from the extremity through the femoral vein was determined from time to time by placing a bull-dog clamp on the vein proximal to the cannula, removing the clamp on the rubber side tube and collecting the blood for a given period of time. Usually no difficulty was encountered because of clotting for at least one and one-half hours after the giving of heparin.

Blood samples and oxygen content. Blood samples were withdrawn under oil and placed under oil in order to avoid contact with air The sample from the femoral vein was obtained through the side arm of the cannula immediately after the total flow from the femoral vein in a unit of time had been determined. Arterial blood was obtained from the femoral artery of the opposite side. Mixed venous blood was obtained from the right side of the heart by puncture. The Van Slyke-Neill manometric apparatus was employed in determining the oxygen content of the samples of blood.

Oxygen consumption. This was determined by the use of a Benedict spirometer with a graphic recording device. Connection was made between the animal and the spirometer either by a tracheal cannula or a specially constructed rubber mask for the mouth.

Cardiac output. The output of the heart was calculated by using the Fick formula which is as follows:

$$\frac{\text{Oxygen consumption cubic centimeters per minute}}{\text{Arterial oxygen content minus venous oxygen content}} = \text{Cubic centimeters of blood flowing through the lungs per minute}$$

RESULTS. In all of the experiments, the administration of heparin was followed by gradually developing shock. It is thought that this was due largely to the heparin as in some instances the bleeding was almost certainly not sufficient to produce circulatory collapse. However, the changes in cardiac output and the appearance of other manifestations of shock enhanced the value of the experiments since they were associated with the local alterations in blood flow in which we were interested.

Temperature changes. The changes in temperature of the three areas studied did not parallel closely the alterations in the flow of blood through the femoral vein. Frequently a decrease in flow from the femoral vein was associated with an elevation of temperature of the area under observation or an increase in flow from the femoral vein was associated with a decrease in temperature of the same area. This inverse relationship was encountered at least once in 1, three of the six experiments in comparing the femoral outflow with the temperature of the foot; 2, five of the six experiments in comparing the femoral outflow with the temperature of

the subcutaneous tissues of the lower leg; and 3, four of the six experiments in comparing the femoral outflow with the temperature of muscular tissue of the thigh.

On comparing the temperature changes of the three areas which were studied, it was noted that no direct relationship existed between them. That is, although in some instances a decrease in temperature was noted in the three localities, in others a decrease in one part was associated with a rise in another. The decrease in the temperature of the foot when the blood flow through the leg diminished was usually greater than that of the subcutaneous tissues of the lower leg and of the muscle of the thigh.

The arterio-venous difference in oxygen content. In all instances in which there were marked alterations in the amount of blood flowing from the femoral vein, changes in the differences in oxygen content of blood from the femoral artery and vein were found. An increase in flow from the leg was accompanied by a decrease in the arterio-venous difference and vice versa. This is shown in charts 1 and 2. In several instances, there was a slight change in flow without any alteration in the arterio-venous difference and in other instances a slight change in the arterio-venous difference without any alteration in flow. In other words, alterations in the blood flow through the leg were not always accompanied by corresponding changes in the arterio-venous difference. In experiment 6 (chart 2) the increase in the arteriovenous difference was almost directly proportional to the decrease in the flow from the leg. The difference in oxygen content of blood from the femoral artery and vein was not always associated with a corresponding difference in oxygen content of samples from an artery and the right side of the heart.

Outflow of blood through femoral vein. In four of the experiments, the outflow of blood from the femoral vein showed a steady decrease throughout the experment. As was stated previously, this can be accounted for in most instances by a drop in the cardiac output probably due to heparin.

The changes in blood flow through the leg roughly parallel alterations in the cardiac output. If it may be assumed for the moment that the cardiac output could be calculated by dividing the oxygen consumption by the difference in oxygen content of the femoral artery and vein instead of that of the artery and right side of the heart, then again the changes in blood flow through the leg roughly parallel alterations in the cardiac output as determined by this method.

The changes in volume flow through the leg have already been compared with alterations in the temperature and in arterio-venous differences in gaseous content.

Oxygen consumption. The total oxygen consumption per unit of time remained practically constant in all of the experiments. This is not surprising since the oxygen consumption is very rarely altered appreciably even in severe states of acute circulatory insufficiency.

Cardiac output. The output of the heart usually declined steadily as the experiment was continued. It is thought that this was due mainly to the heparin and partially to the loss of blood.

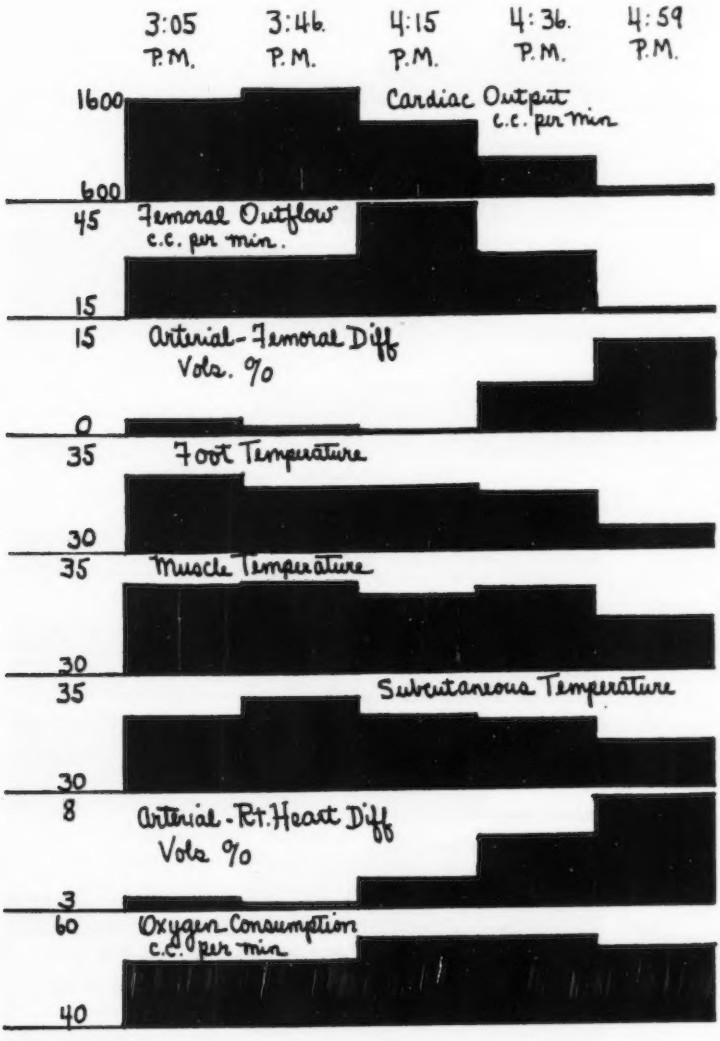

Chart 1. Showing graphically the results of experiment 3

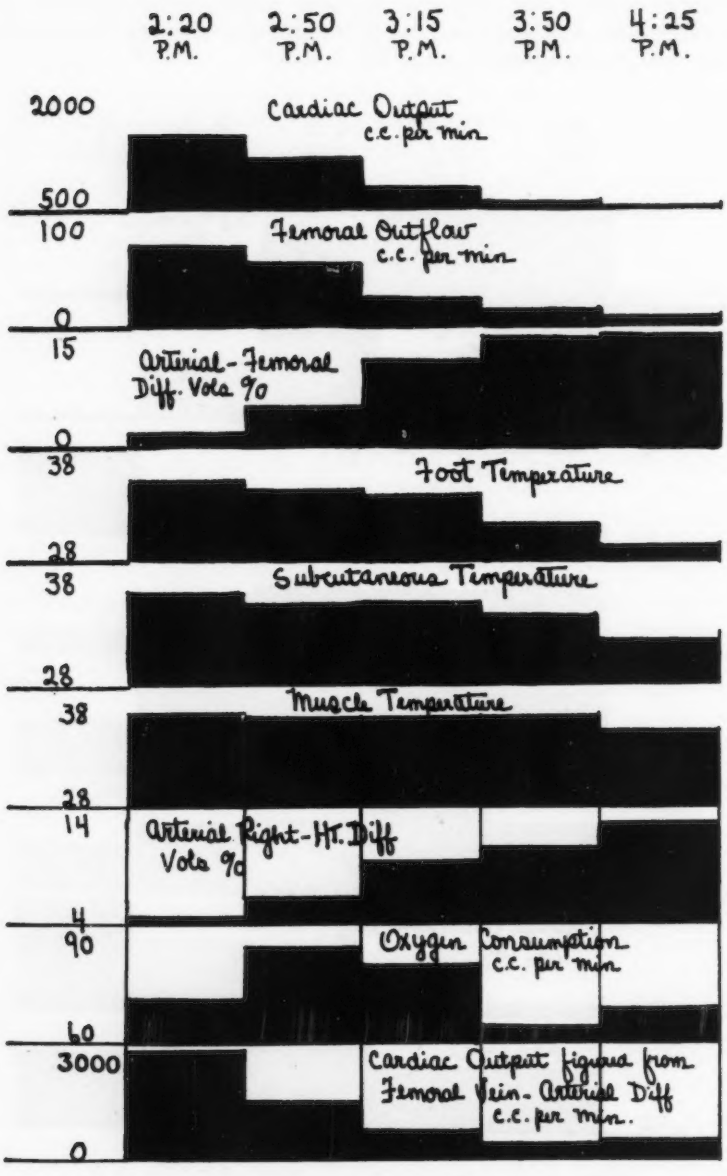

Chart 2. Showing graphically the results of experiment 6

DISCUSSION. The studies which are reported here show that the local changes in the temperature in various parts of the leg do not necessarily serve as an index of the total flow of blood through the entire extremity. It is likely that they do indicate changes in blood flow in the small area in which the temperature was determined. The remarkable constancy of the oxygen consumption of narcotized dogs, whether essentially normal or fairly severely shocked as determined in these and many other experiments leads one to think that the oxygen consumption in local areas does not vary tremendously from time to time. The work of Gaskell which has been mentioned previously indicates that the temperature is not a reliable index of the total flow of blood through a part.

The directional changes in the difference in oxygen content of the femoral artery and vein bear a constant relationshp to changes in the total flow of blood. No proportional relation exists in all instances, as for example, if the flow of blood through the leg decreased from 100 cc. per minute to 75 cc., one would not be justified in assuming that the arterio-venous difference in oxygen content would increase by 25 per cent. Goldschmidt and Light (1925) have called attention to the marked independent variations in oxygen content of the blood from closely adjacent veins of the forearm. Previous unpublished observations by one of us (A.B.) upon both trained and narcotized dogs show that the oxygen content of blood from the femoral vein does vary from time to time, but these variations are not as great as are those of the blood from the superficial veins of the forearm. It cannot be maintained that changes in difference in oxygen content of the artery and vein serve as an accurate index of alterations in flow through the part, but it is believed by using the femoral vein, which drains most of the blood from the leg, that variations in the arterio-venous difference in oxygen content indicate the directions of the change in the blood flow.

It is not surprising that alterations in the total cardiac output and in the flow through the leg usually parallel each other. Neither is it surprising, in using a hypothetical cardiac output obtained by dividing the oxygen consumption by the difference in oxygen content of blood from an artery and the femoral vein, to find that changes in such figures also parallel rather closely alterations in the flow through the leg.

No effort has been made in this work to determine the flow of blood through small areas of tissue except by measuring the temperature but these studies together with determinations of the flow through the entire leg are thought to corroborate the commonly held belief that the blood flow through tissues fluctuates markedly from time to time.

SUMMARY

1. Changes in temperature in any area of the leg do not bear any constant relationship to alterations of *a*, the total flow of blood through the entire extremity, or *b*, the difference in oxygen content of the blood from the femoral artery and vein, or *c*, the total oxygen consumption of the body, or *d*, the output of the heart.

2. It is thought that the temperature of any particular area is an index of the flow through that part.

3. Changes in the difference in the oxygen content of blood from the femoral artery and vein indicate alterations in the flow of blood through the leg but the relationship between these changes is not always proportional.

4. In these experiments alterations in the flow of blood through the leg parallel roughly changes in cardiac output but bear no relationship to the small variations in the oxygen consumption of the body.

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FURTHER DATA CONCERNING THE HISTAMINE SALIVARY SECRETION[1]

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In a previous paper on the action of histamine on salivary secretion (1) it was shown that this substance has a double effect on the submaxillary glands of both cats and dogs. Besides producing a true secretion, it activates some contractile elements in the gland which results in a pressing out of the saliva. A more detailed investigation of this latter phenomenon has been made and the corresponding results are reported here.

Ergotamine. The augmented secretion, obtained when the sympathetic nerve is stimulated after the chorda, has been shown to be due partly to the action of motor fibres and to a lesser extent to the secretory fibres (2). Since there is a similarity between the twofold action of the sympathetic and that of histamine it was considered possible that both might be acting on the same mechanism in the gland. Therefore it was important to study the histamine salivary secretion after the sympathetic nerve had been paralyzed with ergotamine.

Ergotamine methasulphonate, Sandoz preparation, was used for these experiments. The salt was dissolved in distilled water just before using and was injected intravenously. Repeated injections of small doses ($\frac{1}{8}$ to $\frac{1}{4}$ mgm. in a cat, $\frac{1}{2}$ mgm. in a dog) did not prove as effective as a single injection of a bigger dose (4–5 mgm. per kilo body weight).

The sensitivity of different parts of the sympathetic nervous system to ergotamine was found to vary considerably. By stimulating the peripheral end of the cervical sympathetic, following the injection of this drug the first noticeable effect was the disappearance of the sympathetic salivary secretion, later the vasoconstrictor action was abolished and finally the dilatation of the pupil. These facts are in accordance with the observations of Anrep (3) who could see that ergotoxine first paralyzed the motor fibres for the submaxillary gland and later the vaso-constrictor fibres. When the ergotamine effect was fully developed it was found that adrenalin (1 cc. 1/10,000) injected intravenously, did not have its usual pressor or

[1] This research has been carried out under a grant given by the National Research Council of Canada.

secretory effect and in some instances even produced a slight fall of blood pressure.

In all cases the animals were anesthetized with chloralose (0.1 gram per kilo weight), a cannula was then inserted into the submaxillary duct and connected to a Gibbs drop recorder (4) measuring 46 drops in 1 cc. The chorda lingual and cervical sympathetic nerves were cut and blood pressure recorded from the carotid artery. In each experiment a fresh solution of histamine (acid phosphate) was used. It should be noted here

TABLE 1

November 14, 1928. Cat 2.65 kgm. Chloralose

TIME	PROCEDURE	SALIVA	REMARKS
		drops	
11 hrs. 3′	Sympathetic for 30″, coil 11	3	
11 hrs. 9′	Chorda for 30″, coil 12	28	
11 hrs. 10′	Sympathetic for 30″, coil 11	14	
11 hrs. 22′	Chorda for 30″, coil 12	27	
11 hrs. 23′	Histamine $\frac{1}{4}$ mgm.	8	Fall of blood pressure returned in 4′ to normal
11 hrs. 37′	Ergotamine 10 mgm.	0	Slight rise and marked fall in B.P. for 10′ never returned to normal
11 hrs. 45′	Chorda for 30″, coil 12	12	
11 hrs. 46′	Sympathetic for 30″, coil 10	0	No dilatation of pupil. No rise in blood pressure
11 hrs. 49′	Chorda for 30″, coil 12	13	
11 hrs. 50′	Histamine $\frac{1}{4}$ mgm.	4	Small fall in blood pressure
12 hrs. 5′	Suprarenals excised	0	
12 hrs. 14′	Sympathetic for 30″, coil 10	0	No dilatation of pupil. No rise in blood pressure
12 hrs. 16′	Chorda for 30″, coil 10.5	11	
12 hrs. 17′	Adrenalin 1 cc. 1/10,000	0	No rise in blood pressure
12 hrs. 18′	Histamine $\frac{1}{2}$ mgm.	4	Slight fall in blood pressure

that the response of the salivary glands to histamine was not always uniform in different animals. A special investigation of the factors underlying this individual variability is at present being carried out in our laboratory.

The results obtained with ergotamine definitely show that paralysis of the sympathetic nervous system does not abolish the augmented histamine secretion obtained after stimulation of the chorda. In some experiments ergotamine had no effect on the quantity of saliva secreted after histamine

but in others a diminution in secretion was observed. This diminution might be partly attributed to a decreased excitability of the gland in general during the course of the experiment.

The following experiment is quoted as an example in table 1. In all the tables the length of the time of stimulation of the nerve and the distance between the primary and secondary induction coils in centimeters are given. (See also fig. 1.)

One may see from the above table that after complete paralysis of the sympathetic and adrenalin actions, histamine still produced its typical effect. A dose of atropine sufficient to paralyze the secretory action of the chorda tympani on an ergotamized cat diminished but not quite

TABLE 2

November 5, 1928. Cat 2.5 kgm. Chloralose

TIME	PROCEDURE	SALIVA	REMARKS
		drops	
	Ergotamine 6 mgm. previously injected		
1 hr. 30′	Chorda for 30″, coil 12	17	
1 hr. 31′	Sympathetic for 30″, coil 9	0	No dilatation of the pupil
1 hr. 32′	Histamine $\frac{1}{4}$ mgm.	3	
1 hr. 41′	Ergotamine 2 mgm.	0	Fall in blood pressure
1 hr. 47′	Sympathetic for 30″, coil 9	0	Slight dilatation of pupil
2 hrs. 0′	Chorda for 30″, coil 11	18	
2 hrs. 1′	Atropine $\frac{1}{4}$ mgm.	0	
2 hrs. 2′	Chorda for 30″, coil 11	0	
2 hrs. 3′	Histamine $\frac{1}{4}$ mgm.	1	
2 hrs. 6′	Chorda for 30″, coil 11	0	
2 hrs. 7′	Sympathetic for 30″, coil 9	0	Very slow and slight dilatation of the pupil
2 hrs. 11′	Saliva blown back into the duct		
2 hrs. 12′	Histamine $\frac{1}{8}$ mgm.	2	

abolished the histamine action. An example is shown in the experiment below in table 2.

Thus the pressor effect of histamine on the salivary gland is maintained practically unchanged after paralysis of the sympathetic nervous system. On the other hand paralysis of the parasympathetic diminishes, though it does not abolish completely the action of this drug.

As some of the contractile elements of the gland are under the control of the sympathetic, it is assumed that histamine after ergotamine must be acting on these elements at a point peripheral to the endings of the nerve.

Pituitrin. It was important to determine whether the contractile mechanism in the gland, stimulated by histamine, was of a muscular nature. A comparison was therefore made between the action of histamine and

pituitrin, the latter of which produces a contraction of all kinds of smooth muscle. The effect of pituitrin on salivary secretion was very small and could not be compared with the action of histamine. Neither by itself nor after stimulation of the chorda tympani did pituitrin give any noticeable effect, hardly one drop of secretion being pressed through the drop recorder. An example of the striking difference between the action of histamine and pituitrin can be seen in the following experiment in table 3. The same results were obtained in analogous experiments on dogs.

In another form of experiment the chorda was rhythmically stimulated and an even flow of saliva produced. Histamine and pitutrin were injected during this secretion. Again the result was quite different for these two substances, as may be seen from the experiment of December sixth. (Table 4.)

There is quite a marked inhibition of the salivary flow following the injection of pituitrin and a very insignificant one after histamine. This last is probably due to the fall of blood pressure and diminished supply of blood to the gland. Only in the case of a very great fall in the blood pressure after a larger dose of histamine ($\frac{1}{2}$ mgm.) was there a short inhibition of the secretion induced by a rhythmic stimulation of the chorda.

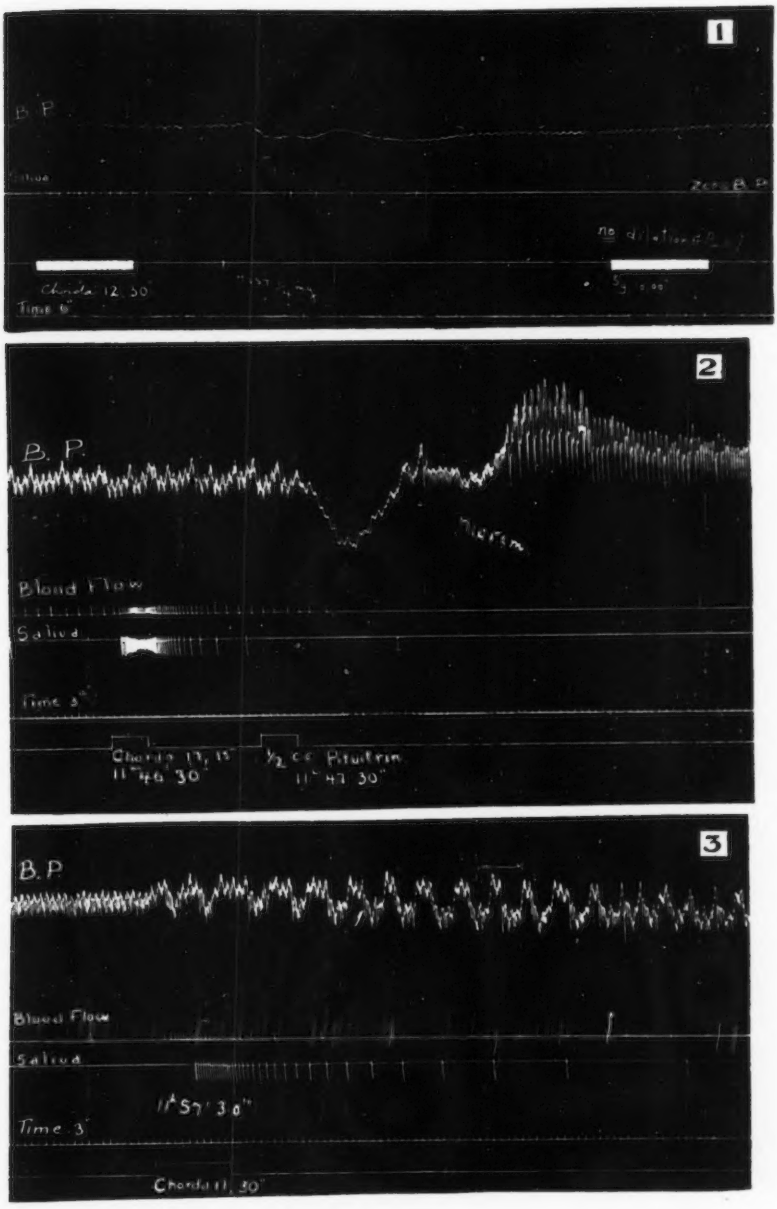
The probable explanation of the inhibitory effect of pituitrin on the salivary flow, induced by a rhythmic stimulation of the chorda tympani, is that it is due to a constriction of the glandular blood vessels. Special experiments on dogs were performed to clear up this problem. These animals, anesthetized with chloralose, were prepared as usual and in addition to the secretion the blood flow through the gland was recorded. For this purpose Gesell's (5) blood volume flow recorder was used.

An example may be cited from one of several analogous experiments. The rate of the blood flow was equal to six strokes of Gesell's apparatus in thirty seconds. Stimulation of the chorda for fifteen seconds gave a profuse flow of saliva and increased the blood flow from six to forty-three strokes in thirty seconds. Injection of $\frac{1}{2}$ cc. pituitrin gave one drop of saliva and altogether stopped the circulation through the gland for nine and a half minutes. A subsequent stimulation of the chorda only increased the rate of blood flow from one stroke in thirty seconds to sixteen

Fig. 1. Cat, chloralose, showing the action of histamine after ergotamine which was injected previously. Stimulation of the cervical sympathetic did not produce any effect.

Fig. 2. Dog, chloralose. Gesell's blood volume flow recorder. Shows the stimulation of chorda followed by intravenous injection of pituitrin. Note the inhibition of the blood flow through the gland and practically absence of the salivary secretion.

Fig. 3. Same dog as on figure 2. Five minutes and 27 seconds have elapsed between figures 2 and 3. Blood flow through the gland almost stopped. On figure 3 is shown the effect of the second stimulation of the chorda tympani after the administration of pituitrin.


1
B. P.
Saliva
Chorda 12, 30
Time 5"
2
B. P.
Blood Flow
Saliva
Time 5"
Chorda 12, 15
11" 46' 30"
½ c.c. Pituitrin
11" 47' 30"
3
B. P.
Blood Flow
Saliva
11" 57' 30"
Time 5"
Chorda 11, 30"

Figs. 1 to 3

strokes, and the secretory effect was much less than that observed before the injection of pituitrin. A second stimulation of this nerve still did not give the full amount of secretion and vaso-dilatation. It was only after the third stimulation of the chorda that the secretory and vaso-dilator effects were practically equal to that obtained before the injection of pitui-

TABLE 3

December 10, 1928. Cat. Decerebrate

TIME	PROCEDURE	SALIVA	REMARKS
		drops	
11 hrs. 52′	Chorda for 30″, coil 16	29	
11 hrs. 53′	Histamine ¼ mgm.	14	Marked fall in blood pressure
12 hrs. 7′	Chorda for 30″, coil 16	28	
12 hrs. 8′	Pituitrin 1 cc. (2½ units Burroughs Wellcome pituitary extract)	1	Rise in blood pressure

TABLE 4

December 6, 1928. Cat 2.5 kgm. Chloralose

TIME	PROCEDURE	RATE OF SALIVARY SECRETION EVERY 30″ IN DROPS	REMARKS
12 hrs. 31′	Rhythmic stimulation of chorda, coil 21	30″ before injection of pituitrin—4	
12 hrs. 31′ 30″	Pituitrin ½ cc. (1¼ units Burroughs Wellcome pituitary extract)	30″ after injection of pituitrin—2 1′ after injection of pituitrin—0 1′ 30″ after injection of pituitrin—2	Rise in blood pressure
1 hr. 14′	Rhythmic stimulation of chorda, coil 18.5	30″ before injection of histamine—5	
1 hr. 14′ 30″	Histamine ¼ mgm.	30″ after injection of histamine—4 1′ after injection of histamine—5 1′ 30″ after injection of histamine—4	Fall in blood pressure

trin. Thus though pituitrin acts very powerfully on the blood vessels it failed to press out saliva from the gland. (See figs. 2 and 3.)

Kogan, Ponirovski and Raiski (6) found that the intravenous injection of pituitrin caused an inhibition of the secretion activated by pilocarpine in a dog with a permanent fistula of the mixed salivary gland. This diminution of the salivary flow was very prolonged lasting for twenty-five

minutes after 1 cc. pituitrin. The conclusion reached by these workers was that these two substances, pilocarpine and pituitrin, in so far as their effect on the salivary secretion was concerned, were antagonistic to each other. But the experiment quoted above, in which the blood flow through the gland was measured, shows that in discussing the antagonsim between pilocarpine and pituitrin the circulation in the gland must be an important factor. In the experiments of Kogan, Ponirovski and Raiski the inhibition of the pilocarpine secretion produced by pituitrin could be due, not to the secretory antagonism between the two substances, but to a long continued shortage of blood supply to the gland.

TABLE 5

January 20, 1928. Dog. Chloralose

TIME	PROCEDURE	SALIVA	BLOOD FLOW	BLOOD PRESSURE
		drops		*mm.* Hg
12 hrs. 21′	Adrenalin 1 cc. 1/10,000	0	Inhibition	Rise from 130 to 200
12 hrs. 27′	Sympathetic for 15″, coil 12	14	Marked inhibition	No change
1 hr. 27′	Chorda for 15″, coil 15	113*	Marked acceleration	No change
1 hr. 32′	Adrenalin 1 cc. 1/10,000	4	Slight inhibition	Rise from 118 to 160
1 hr. 34′ 30″	Sympathetic for 15″, coil 12	14	Marked inhibition	Rise from 116 to 124
1 hr. 56′	Pituitrin 1 cc.	1	Very marked inhibition	Fall from 112 to 5 then rose to 188

* Long continued secretion.

Adrenalin. Since adrenalin substitutes the effect of sympathetic stimulation on the salivary glands, a few experiments were performed with this substance and its action was compared with that of pituitrin.

A dose of adrenalin may be found which by itself is not large enough to activate the secretion of saliva but which will, after stimulation of the chorda, give an augmented effect. However, in considering this augmented effect it must not be thought of as a pure "pressing out," the possibility of a true augmented secretion being denied. Some experiments indicated that repeated injections of a small dose of adrenalin gave an augmented secretion without previous stimulation of the chorda. While subminimal doses of adrenalin may produce a secretion in an activated gland, it was found that pituitrin, under the same conditions, had practically no effect. The rise of blood pressure after administration of the two substances was approximately equal. See table 5.

Thus when adrenalin alone did not produce any visible effect it gave a secretion if the gland had previously been activated. Pituitrin, on the other hand, acted neither after stimulation of the secretory nerves, nor in a non-activated gland. For an example of pituitrin action, after stimulation of the sympathetic see table 5, and after stimulation of the chorda see table 3.

DISCUSSION. The results of this investigation show that histamine was acting on the submaxillary gland after complete paralysis of the sympathetic secretory nerve by ergotamine, this substance only slightly diminishing the histamine effect. It has also been noted, in some recent experiments as well as in a previous paper, that histamine may have a secretory action after paralysis of the parasympathetic nerve by atropine. However, it must be emphasized that this secretory effect was very much less than that obtained after ergotamine. The fact that atropine had such an influence on the histamine secretion may be due to its causing a general poisoning of the gland, since, in addition to paralyzing the chorda, this drug not infrequently diminished the action of the sympathetic. Large doses may even completely abolish the action of this nerve. On the other hand ergotamine affected only the sympathetic nervous system and did not interfere very much with the action of the chorda.

It has been proved that there are fibres for some contractile elements of the submaxillary gland in the sympathetic nerve. Therefore if histamine is partly acting on these elements it must be at a point peripheral to that at which the sympathetic endings are paralyzed by ergotamine. However, as the histamine action is diminished after a dose of atropine sufficient to paralyze the chorda, there is a possibility that it may act on the glandular elements innervated by this nerve as well.

This question of the nature of the contractile elements which are responsible for the motor phenomena produced by the sympathetic nerve and by histamine has been dealt with in a conjoint paper with Doctor Babkin.

SUMMARY

1. Paralysis of the sympathetic nerve by ergotamine did not abolish the augmented histamine secretion though in some cases it diminished it.

2. Histamine acted slightly on the salivary secretion after both ergotamine and atropine, though the latter drug lessened its effect very considerably.

3. A comparison of the action of histamine and pituitrin showed that these two substances were not acting in the same way on the submaxillary gland. This is evidence for the view that histamine is not acting on contractile elements in the gland which are of a muscular nature.

4. Subminimal doses of adrenalin which alone did not produce a secretion may, in an activated gland, produce a small flow of saliva.

5. Pituitrin did not give a secretion after previous stimulation of either of the secretory nerves, despite the fact that it has a very marked motor action on the smooth muscle of the glandular blood vessels.

It is a great pleasure to express my thanks to Doctor Babkin for advice and direction throughout this work.

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THE COMPOSITION OF THE SALIVA IN DIFFERENT PHASES OF THE SECRETION AND BY REPEATED STIMULATION

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In a reflex action of a stimulus on the salivary glands it is essential to discriminate between two phases. The first phase includes the latent period and the period of activity of the given stimulus. The second phase is that of the after effect, i.e., when the stimulus no longer acts on the receptor surface, but the gland still continues to secrete for a certain period of time. Many data have been collected relating both to the quality and quantity of the saliva of the different salivary glands in the dog under different stimuli, during the first phase. Yet hardly more than a general statement is made; that the after flow during the second phase of the secretion is directly proportional to the strength of the initial stimulus. A study of this problem is prerequisite to the fuller understanding of the complicated mechanism of the innervation of the salivary glands, since during the second phase the gradually dying traces of irritation of the receptors, the fading excitation in the centres, and the changing irritability of the peripheral neuro-cellular apparatus are dealt with.

METHODS. Experiments were performed on a dog with a permanent parotid fistula, the animal was in good condition and kept his weight during the investigation. The saliva was collected by the classical method used by Pavlov. The intrusion of unusual conditioned stimuli upon the salivary secretion was avoided by every possible scruple.

Certain improved methods of determining the organic substances and ash of the saliva were devised. The data of previous investigators, Heidenhain, Langley, Wulfson, Sellheim, Babkin and others vary considerably in the percentage of inorganic salts. Those using platinum crucibles obtained the highest figures for salts, demonstrating the advantage of platinum over porcelain. To prevent undesirable loss of salts Babkin (1) added one to three drops of a saturated solution of ammonium nitrate to aid combustion in a porcelain crucible. Not desiring to add foreign chemicals, and porcelain crucibles being the only kind obtainable, a method of combustion was devised which reduced error to a minimum.

A small electric furnace operated by the 110 volt direct current, with a

capacity of twelve crucibles was constructed. An alundum muffle was wound with chromel wire and placed in a loose metal sheath; the intervening space tightly packed with asbestos fibre, and the ends sealed with a mixture of alundum cement and fire clay; a rheostat was included in the circuit to control the current. Oxygen was introduced through one end and permitted to escape at the other through a hole in the door. Both temperature and oxygen flow could be altered by controls to any degree of intensity. A comparison of the melting points of the various salts contained in saliva shows that the chlorides are affected first at slightly over 600°C. Experimentally 450°C.–540°C. completely removed all traces of carbon and therefore the latter point was not exceeded. The

TABLE 1

EXPERIMENTAL PROCEDURE	PER CENT OF SOLIDS	PER CENT OF ORGANIC	PER CENT OF ASH
Burned over flame for 4 minutes	1.04	0.58	0.46
Burned over flame for 30 minutes	1.02	0.39	0.63
Porcelain crucibles in furnace 2 hours	1.04	0.34	0.70
Porcelain crucibles in furnace 5 hours	1.03	0.33	0.70
Platinum crucibles in furnace 30 minutes	1.03	0.27	0.76

TABLE 2

AMOUNT OF SALIVA	PER CENT OF SOLIDS	PER CENT OF ORGANIC	PER CENT OF ASH
cc.			
2.0	0.83	0.26	0.57
1.0	0.83	0.25	0.58
1.0	0.84	0.24	0.60
0.8	0.825	0.255	0.59
0.5	0.82	0.22	0.60
0.3	0.865	0.13	0.735
0.2	0.80	0.10	0.70
0.1	0.80	0.10	0.70

following experiment illustrated the relationship between ashing in a bunsen flame, and in the furnace, different kinds of crucibles being employed. Platinum is the most ideal on account of its rapid and uniform transmission of heat (table 1).

A thermostatic calibration of the furnace showed that it could be controlled with accuracy between 450°C. and 600°C. With regard to the oxygen flow, it is essential to use a large volume at first to aid the early carbonization and elimination of the organic residue (approximately one litre and a half per minute for the first hour). The time of heating necessary varies in direct proportion to the amount of organic matter present; empirically from four to five hours for parotid and still longer for sub-

maxillary saliva sufficed to remove all carbon. The quantity of saliva most suitable for analysis was found to be 1 cc. as may be observed from the following table (table 2).

The objection to determinations made with 2 cc. or more is that the greater amount of organic matter present in the crucibles makes elimination correspondingly longer and more difficult, the flow from the gland does not always provide that quantity, and the final results are the same (when allowance is made for slight variation due to the error of the method).

TABLE 3

The table appended shows typical examples of parotid saliva secreted in response to stimulation by bread and meat powder; 0.25 per cent HCl and 5 per cent NaCl.

DATE	STIMULATION	AMOUNT OF SALIVA	PER CENT OF SOLIDS	PER CENT OF ORGANIC	PER CENT OF ASH
		cc.			
February 11	Bread and meat powder	2.4	1.03	0.28	0.75
February 20	Bread and meat powder	2.4	1.02	0.28	0.75
February 18	0.25 per cent HCl	2.5	1.00	0.28	0.72
February 18	5 per cent NaCl	2.4	0.99	0.30	0.69

TABLE 4

Composition of the parotid saliva collected in one minute during the action of the stimulus

DATE (1929)	STIMULUS	AMOUNT OF SALIVA	PER CENT OF SOLIDS	PER CENT OF ORGANIC	PER CENT OF ASH
		cc.			
May 29	Bread and meat powder*	2.4	1.14	0.39	0.75
May 15	0.25 per cent HCl†	2.6	1.09	0.29	0.80
April 19	0.5 per cent HCl‡	2.6	1.67	1.00	0.67
May 17	5 per cent NaCl§	2.6	1.01	0.10	0.91

* Fed for 5 seconds each at 0, 20, 35, and 55 seconds.
† Five cubic centimeters at 0, 20, 40 and 60 seconds.
‡ Injection at 0, 20 and 40 seconds.
§ Injection at 0, 20, 40 and 60 seconds.

Composition of the saliva in different phases of the secretion. In previous work on the salivary glands this investigation was originated by an occasional observation on a dog with a permanent parotid fistula. The secretion was stimulated by different substances for one minute and the saliva was collected for one minute and thirty seconds. Three feedings of the chemical were given, at 0 second, 30 seconds and 60 seconds respectively. The result was quite unexpected, for with such experimental procedure no significant difference in the amount of organic matter was detected after stimulation with hydrochloric acid and sodium chloride (table 3).

Wulfson (2) and Sellheim (3) have shown that the parotid saliva secreted in response to 0.5 per cent hydrochloric acid is more than twice as rich in the organic fraction as that from 10 per cent sodium chloride; conse-

TABLE 5

Composition of the parotid saliva during different phases of the secretion

The first sample obtained in each experiment was discarded.* Feeding at 0, 20, 40 and 60 seconds was the routine and in case of bread and meat powder the duration was 5 seconds each at 0, 20, 35, 55 seconds.

DATE	STIMULUS DURING 1 MINUTE	INTERVAL BETWEEN STIMULUS	FIRST MINUTE				SECOND MINUTE				THIRD TO SEVENTH MINUTE INCLUSIVE			
			Amount	Per cent of solids	Per cent of organic	Per cent of ash	Amount	Per cent of solids	Per cent of organic	Per cent of ash	Amount	Per cent of solids	Per cent of organic	Per cent of ash
			cc.				cc.				cc.			
May 29	Meat and bread powder	12′	2.4	1.14	0.39	0.75	0.9	1.21	0.51	0.70	0.9	0.64	0.25	0.39
		45′†	2.5	1.07	0.32	0.75	0.7	1.25	0.50	0.75	0.8			
		11′	2.6	1.33	0.60	0.73	1.3	1.46	0.80	0.66	1.0			
			2.7	1.23	0.45	0.78	1.4	1.21	0.50	0.71	1.0			
Average			2.6	1.19	0.44	0.75	1.1	1.28	0.58	0.70	0.9	0.64	0.25	0.39
April 19	0.5 per cent HCl	22′	2.6‡	1.67	1.00	0.67	2.5	1.70	1.13	0.57	1.4	0.78	0.26	0.52
		15′	2.8	1.44	0.70	0.74	1.8	1.52	0.89	0.63	1.3	0.58	0.18	0.40
			2.8	1.24	0.64	0.60	2.0	1.37	0.75	0.62	2.5	0.64	0.14	0.40
Average			2.7	1.45	0.78	0.67	2.1	1.53	0.92	0.61	1.7	0.67	0.19	0.44
May 15	0.25 per cent HCl	7′	2.6	1.09	0.29	0.80	1.5	1.44	0.62	0.82	Not determined			
		30′§	2.8	1.12	0.39	0.73	1.1	1.40	0.64	0.76				
		9′	2.8	1.44	0.68	0.76	2.2	1.62	0.84	0.78				
			2.6	0.90	0.20	0.70	1.4	1.06	0.28	0.78	1.1	0.60	0.05	0.55
Average			2.7	1.14	0.39	0.75	1.5	1.38	0.59	0.78				
May 17	5 per cent NaCl	10′	1.9	0.92	0.10	0.82	1.1	0.98	0.16	0.82	0.3	0.91	0.30	0.61
		30′**	2.0	0.87	0.19	0.68	1.0	1.12	0.31	0.81	0.3			
		11′	1.8	1.02	0.12	0.90	1.1	1.12	0.26	0.86	0.3			
			1.9	1.04	0.24	0.80	0.8	1.18	0.37	0.81	0.2			
Average			1.9	0.96	0.16	0.80	1.0	1.1	0.22	0.82	0.3	0.91	0.30	0.61
May 1	10 per cent Na_2CO_3	9′††	1.8	0.92	0.18	0.74	0.9	0.95	0.20	0.75				
		30′	1.4	0.82	0.16	0.66	0.4	1.00	0.33	0.67				
		7′	1.6	1.06	0.28	0.78	0.7	1.18	0.42	0.76				
			1.7	1.00	0.22	0.78	0.8	1.07	0.32	0.75				
Average			1.6	0.95	0.21	0.74	0.7	1.0	0.32	0.73				

* It was early found that the first sample of the secretion almost invariably contained an excess of solids due to the fact that the gland had been previously resting.

† Saliva collected in the 45-minute rest period amounted to 9 cc. solids 0.615; organic 0.155; ash 0.460.

‡ This sample was the first of the experiment.

§ Flow amounted to 5.4 cc. in the 30-minute interval solids 0.55; organic 0.01; ash 0.54.

** Only 0.3 cc. was produced during above interval (not enough for separate analysis).

†† This sample was the third of the experiment.

quently when their method was employed (i.e., collecting the saliva during one minute when the stimulus acted on the mouth cavity) typical "acid" and "salt" secretion with marked difference in the percentage of

organic substance was obtained, although 0.25 per cent hydrochloric acid and 5 per cent sodium chloride were employed.

Table 4 contains typical data obtained under similar conditions; the amount of saliva is almost identical. A theoretical explanation may be employed to account for the different response apparent in tables 3 and 4. Since in the first case the stimulus acted at intervals for one minute and saliva was collected for one minute and thirty seconds and in the second case the time of collection of the saliva practically coincided with the time of action of the stimulus, it would seem a priori that the saliva of the after-flow varies in composition from that secreted during the stimulation. To further elucidate this fact, experiments were performed in which three separate specimens of saliva were collected: *a*, the first minute during stimulation; *b*, after-flow during second minute; *c*, after-

TABLE 6

Parotid saliva, stimulus bread and meat powder given at 0, 20, 35, 55 seconds for 5 seconds each

Saliva collected during first minute and successive five minutes. The first sample was discarded.

NUMBER OF SAMPLE	INTERVAL	FIRST MINUTE				SUCCESSIVE FIVE MINUTES			
		Amount	Per cent of solids	Per cent of organic	Per cent of ash	Amount	Per cent of solids	Per cent of organic	Per cent of ash
		cc.				cc.			
2		1.4	0.70	0.16	0.54	0.7	0.76	0.20	0.56
3	8′	1.6	0.76	0.17	0.59	1.0			
4	30′*	1.5	0.83	0.19	0.64	1.0	0.70	0.20	0.50
5	8′	1.5	0.78	0.20	0.58	0.9			

* Less than 0.5 cc. of saliva secreted during half-hour interval.

flow from third to the seventh minutes inclusive. The composition of the saliva was determined separately in each sample. Since the flow was sometimes very scanty in the last period, the secretion of the same experiment was mixed and an average figure obtained. (Table 5.)

The effect of different agents, bread and meat powder, hydrochloric acid, sodium chloride and sodium bicarbonate, is shown in the different phases in single experiments as well as in average data. In every instance the second period shows a greater percentage of organic than the first minute. Since the amount of ash varies but slightly in the first and second minutes the chief increase in the total solids is due to a marked rise in the amount of organic elements secreted during the second minute. The secretion collected from the third to seventh minutes, was characteristically poorer in total solids and especially in organic constituents. The

fall was chiefly due to the scanty secretion. It is very doubtful whether the secretion in the third period after feeding was so called "termoregulation" or "excitation" saliva (4) (5). First of all the secretion usually stopped, and secondly in most of the experiments there was no panting. This saliva of the third period (third to seventh minute), however, resembles the composition of "excitation" saliva. If the initial stimulation with bread and meat powder was not strong enough to produce a long continued secretion the content of organic matter in the after-flow was practically the same as during the action of the stimulus. (Table 6.)

By the above arrangement of the experiment in the second period of the flow a mixed saliva was obtained composed of different phases of the after-flow. The higher content of solids of the second minute was reduced by the lower amount in the successive minutes of collection.

Variations in the composition of the saliva by repeated stimulation. The above described experiments with long continued secretion graphically portray the fact that different impulses reach the gland during the first, second and successive five minutes after the stimulation. To more clearly understand the mechanism of this phenomenon it was important to determine the effect of two successive stimuli on the gland. Several experiments were performed with repeated stimulation of both the parotid and submaxillary glands. All experiments of my predecessors were performed on dogs with permanent fistulae of the mixed glands or of the submaxillary gland in acute experiments. The viscosity of the saliva was usually the only property determined. Lubimoff (6) found by repeated feeding of a dog with a permanent fistula of the mixed glands a fall in viscosity of the saliva; and after repeated injections of hydrochloric acid, a rise. Stepanoff (7) obtained different results. His data show that the second feeding immediately following the first (each feeding lasting one minute) or after an interval of one, two or three minutes, a saliva more viscid than the first feeding was produced. Especially great differences were noted when the second feeding followed the first feeding three to four minutes after. By increasing the interval between the first and second feeding the difference of viscosity gradually diminished and between fifteen to twenty minutes was the same. By further increase in the interval the difference again appeared, reaching a maximum between thirty to thirty-five minutes. This second increase in the viscosity was far less than the first rise (i.e., between three to four minutes). Stepanoff also observed that if a rejectable substance (5 per cent hydrochloric acid, pepper, sand) was interjected between two feedings the viscosity of the saliva secreted on edible substances was greater than when the previous rejectable stimulus was omitted. The same change occurred when a loud noise (imitation of thunder which alone will cause no secretion) was used instead of rejectable material. He ventures the hypothesis that in a

great degree the variation in composition of the saliva described above originates in the central nervous system. Maevsky (8) confirmed Stepanoff's data, and further, noted not only an increase in the viscosity of the saliva from repeated feedings but similar results from injection of 0.5 per cent hydrochloric acid. Maevsky obtained on a dog under anesthetic, salivary secretion of increased viscosity by repeated stimulation of the peripheral end of the chorda tympani. He concluded that the effect was chiefly due to the change of excitability of the peripheral part of the reflex arc, and not to various states of the central nervous system as Stepanoff believed. He assumed that the changes of the viscosity of the saliva are identical to the changes of the organic matter in it.

Table 7 contains data portraying the influence of the first feeding on the composition of the saliva of the second feeding; the interval between the two varying from zero to thirteen minutes and the saliva being collected for one minute and thirty seconds. When this procedure was employed the second feeding in all instances produced secretions (beginning with one minute interval) in which the organic content was either less or practically the same as that in the first feeding. The difference was especially marked in those cases when the first feeding gave a relatively high percentage of solids. It is well proven by experiment that the saliva obtained by the first stimulation of the parasympathetic nerve by an induced current, or after a long period of rest is richer in organic elements than the secretion of a recently activated gland. It is probable that in a dog with a permanent salivary fistula the first nervous impulse reaching the salivary gland after a rest period usually activates a secretion with greater organic content than subsequent samples; provided the animal does not lick himself before the experiment or if there is little or no flow during fixation of the funnel to the animal. The first feeding may then be regarded as the first stimulus. For this reason in all later experiments the first sample was discarded; also the interval between the stimuli was varied. As is evinced in table 5, after a rest interval of thirty to forty-five minutes there was usually an increase both in the solid and organic matter present in the saliva stimulated by bread and meat powder, 0.25 per cent hydrochloric and 10 per cent sodium bicarbonate. In the case of 0.5 per cent hydrochloric there was a gradual decrease in the solid and organic parts probably due to the exhaustion of the gland. The thirty minute interval increased the content of solids in saliva secreted after injection of 5 per cent sodium chloride but a marked increase of organic matter was only seen after the eleven minute interval.

Quite different relations were obtained when there was no interval between the first and second feeding; then the second sample was higher in organic elements than the first. It appears that in these experiments the conditions of the activity of the salivary glands are to a certain degree

analogous to those observed in the first minute of the after-flow. Not all our experiments differ in arrangement from those of Stepanoff (Cf. tables 5 and 7). Notwithstanding this fact our results varied from his. A possible explanation of this discrepancy could be that the activity of

TABLE 7

First and second feedings with meat and bread powder during one minute

Saliva collected from permanent parotid fistula during 1 minute, 30 seconds.

DATE	FEEDING NUMBER	INTERVAL	AMOUNT	PER CENT OF SOLIDS	PER CENT OF ORGANIC	PER CENT OF ASH
			cc.			
June 16	1		2.4	0.92	0.29	0.63
	2	0	2.5	1.06	0.42	0.64
June 17	1		2.6	1.11	0.40	0.71
	2	0	2.5	1.13	0.46	0.67
June 19	1		2.4	0.95	0.23	0.72
	2	1′	2.5	0.94	0.26	0.68
June 10	1		2.5	0.99	0.35	0.64
	2	3′	2.5	0.96	0.35	0.61
June 14	1		2.6	1.32	0.59	0.73
	2	3′	2.5	1.06	0.46	0.60
February 15	1		2.5	1.21	0.54	0.67
	2	5′	2.4	0.93	0.26	0.67
February 20	1		2.4	1.305	0.53	0.775
	2	6′	2.5	1.025	0.285	0.740
February 11	1		2.5	1.22	0.49	0.73
	2	7′	2.4	1.03	0.28	0.75
February 8	1		2.4	1.29	0.56	0.73
	2	9′	2.4	1.10	0.42	0.68
February 18	1		2.5	1.26	0.47	0.79
	2	9′	2.5	1.05	0.38	0.67
February 22	1		2.4	1.08	0.40	0.68
	2	13′	2.4	1.085	0.465	0.62

different glands was studied; (mixed glands in the case of Stepanoff and the parotid gland in our experiments). But the most obvious explanation is that Stepanoff and others determined the viscosity of the saliva only, and omitted the more detailed examination of the composition of the

saliva. May changes in the viscosity of the saliva always reveal proportional variation in the organic fraction? Special experiments disclose no harmonious relationship. Since parotid saliva does not contain mucin, acute experiments were performed on dogs' submaxillary glands to study comparatively the relationship between viscosity and total organic matter. There is one striking example in table 8.

In this experiment the conditions under which the three samples were collected varied. Sample 1 was obtained by rhythmic stimulation of the chorda tympani with a weak current for seven minutes. The collection of sample 2 required more than twenty-one minutes and a stronger current since the chorda tympani nerve was gradually weakening; consequently less saliva was collected. Sample 3 was obtained by a moderate current and in almost the same time as no. 1, but after a rest period of one hour and thirty-seven minutes. In the above experiment the discrepancy between organic content and viscosity is evident. The saliva

TABLE 8

Dog. 7 kgm. Dial 6.75 cc. Right submaxillary gland. Rhythmic stimulation of the chorda lingual nerve

NUMBER OF SAMPLE	TIME	COIL	AMOUNT OF SALIVA	PER CENT OF SOLIDS	PER CENT OF ORGANIC	PER CENT OF ASH	VISCOSITY	
							Just after collection	4 hours later
		cm.	*cc.*					
1	11.34′ to 11.41′	19	2.6	1.58	1.10	0.48	12′ 49″	2′ 05″
2	11.52′ to 12.13′	19–10	1.5	1.76	1.24	0.52	1 hr. 2′ 15″	3′ 34″
3	1.50′ to 1.58′	15–13	2.8*	1.98	1.42	0.56	15′ 50″	

* This sample was of a milky, opalescent color due to long period of rest.

richest in organic was no. 3; that obtained after an interval of one hour and thirty-seven minutes. However, in sample 2 the viscosity was four times as great, with an intermediate organic total. If the samples were left in glass cylinders plugged with cotton wool the viscosity quickly diminished so that four hours after the samples had been obtained the viscosity of samples 1 and 2 was nearly equal. The great viscosity of sample 2 was not due to blocking of the viscosimeter[1] by particles of desquamated epithelium, etc., but to the innate composition of the saliva since the same sample was passed through twice. Therefore the viscosity must not be regarded as an exact replica of the organic content; only in a very general way can comparison be made between the two. There are probably other factors which govern the viscosity of a colloidal solution such as the submaxillary saliva.

[1] The viscosimeter is composed of a small funnel which tapers into a capillary tube of uniform diameter which permits 1 cc. of distilled water to pass through in 7 seconds. The same instrument was used for all determinations.

DISCUSSION. A secretory organ such as the parotid gland of a dog receives different impulses during the action of a stimulus and during its after-effect. Two or even more factors may be involved in this phenomenon.

1. With the subsidence of impulses arriving from the periphery the state of excitation of the salivary centre (that is, the central nervous station in general for this reflex arc) may change. Different impulses, or ones of varying strength to different secretory elements may act on the salivary glands. Stepanoff's experiments showing dissimilarity in the viscosity of the saliva when the surroundings were changed (conditioned stimuli) are suggestive of this explanation. Volborth's (9) results also support this explanation. By repeated feeding with bread for two hours he obtained 152.4 cc. of saliva from a dog with a submaxillary fistula, and observed a fall of 62 per cent in the organic content at the 46th sample. When he gave the dog milk the percentage of organic constituents rose 141 per cent although the secretion was less (46th sample, bread 4.4 cc. organic 0.272. Milk 2.0 cc., organic 0.655).

2. The change in the composition of the saliva in the after-effect of the stimulus may also be due to changes on the periphery. Possibly in the after-effect the salivary centre sends impulses of varying strength to the secretory elements and to the blood vessels. The tonus of the blood vessels which is diminished during the action of the stimulus regains its former parity, the vessels gradually contract and this plays an important part in the diminished flow of saliva. If the impulses to the secretory elements are still strong this shortage of blood supply will cause a rise in the content of organic matter (this is evident in table 5) also when the secretory impulses weaken the organic fraction falls rapidly (third to seventh minute). Without further investigation it is impossible to state which mechanism controls the after-flow of saliva, but in the case of the positive influence of the first feeding on the composition of the saliva of the second feeding, the state of the central nervous system probably plays a predominant part.

SUMMARY

1. The parotid saliva of a dog with a permanent salivary fistula secreted during stimulation varies in composition from that of the different phases of the after-flow.

a. The percentage of organic matter in parotid saliva obtained by different stimuli is higher in the second minute after-flow than in the secretion collected during the first minute of feeding.

b. The organic and inorganic content of the after-flow of the successive five minutes is greatly reduced.

2. The content of organic substances in the parotid saliva of the second feeding is greater only when the interval between the first and the second feeding is less than one minute.

3. There is no exact proportionality between the amount of organic substances in the dog's submaxillary saliva and its viscosity.

4. Certain improved methods of determining the organic and salt fraction were employed.

My thanks are due to Dr. B. P. Babkin for his advice during the course of this work.

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THE EFFECTS OF ASCARIS EXTRACT ON CIRCULATION AND RESPIRATION

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In a previous communication (1928) the authors were able to show that an aqueous extract of Ascaris lumbricoides produced a marked increase in the tonicity of smooth muscle. During these investigations one of the authors showed respiratory nervous and circulatory disturbances similar to those reviewed by Coventry and Taliaferro (1928). The symptoms were so severe that investigations were undertaken to determine the changes occurring in heart rate, blood pressure, rate and depth of respiration, and the volume of some of the organs of various laboratory animals. The animals used for the most part were dogs which were depressed by small subcutaneous injections of morphine sulphate and anesthetized with ether. The blood pressure was obtained from the carotid artery by means of a mercury manometer. The respirations were obtained by means of a tambour which was connected to a rubber bag surrounding the chest of the animal. Plethysmograph volume changes of the spleen and of the intestines were recorded simultaneously with the blood pressure and respiration. A protein free extract prepared from dried pig ascarids, according to the method previously described (Herrick and Emery, 1929), was used in this study. This extract was isotonic and before injecting it was neutralized by means of sodium hydroxide to a pH of 7.4. In the dog experiments all injections were made into the femoral vein. To avoid toxic effects from the extract the large doses were not injected until all the smaller reactions had been obtained. In order to give a larger volume for the small injections the ascaris extract was diluted with physiological salt solution so that 5 cc. contained one cubic centimeter of the extract. We have taken this into consideration in making table 1, and the dosages shown are based on the undiluted extract.

EFFECT ON CIRCULATION. The most noticeable effect of the ascaris extract was the marked drop in blood pressure which occurred without exception in the seventy records obtained from the six dogs studied. The fall of blood pressure was correlated with the amount of extract given. In a dog weighing 26 kilo (no. 2) four small doses of one-tenth cubic centi-

meter each were injected into the femoral vein. The average blood pressure before the injection was 128 mm. and after 109 mm. or an average fall of 19 mm. of mercury. When five cubic centimeters of the ascaris extract were given at a single injection the blood pressure changed from 126 mm. to 52 mm. or a drop of 74 mm. of mercury. In this same dog three cubic centimeters, injected in the same manner into the femoral vein, caused a change of blood pressure from 134 mm. to 44 mm. or a drop of 90 mm. of mercury. Thus, the fall in blood pressure is not in a definite ratio to the amount of extract given but as a general rule the larger the amount injected the greater was the fall in blood pressure. This is further evident by comparing ten small injections taken at random from the data to ten large injections similarly chosen. The average fall in blood pressure obtained for the ten small injections was 21 mm. as compared to a fall of 43 mm. of mercury for the large injections. The return to normal was

TABLE 1

Effects produced by injecting protein free ascaris extract into the femoral vein of dogs under ether anesthesia

DOG NUMBER	WEIGHT	NUMBER OF INJECTIONS	DOSAGE-RANGE	RESPIRATIONS—AVERAGE			HEART RATE AVERAGE			BLOOD PRESSURE	
				Before	After	σ of difference	Before	After	σ of difference	Before	After
	kgm.		*cc. per kgm.*								
1	19	6	0.01 –0.13	54	62	5.7	188	172	18.2	70	40
2	26	16	0.004–0.19	75	75	3.0	166	170	12.7	130	91
3	16	5	0.015–0.06	42	42	1.7	185	177	15.9	93	68
4	17.5	16	0.014–0.06	70	71	6.3	155	173	18.0	95	65
5	5	16	0.02 –0.20	37	41	4.8	192	162	27.4	74	34

quite rapid for small and medium reactions but somewhat delayed when large amounts of the extract were given. A study of fifty records showed the average time for complete return of the blood pressure to the height before injecting the ascaris extract was fifty-six seconds.

The heart rate in each animal after giving the ascaris extract showed small changes which were not correlated with the size of the injection and one could not predict whether the rate would increase or decrease. The heart rates shown in table 1 were taken from the time the blood pressure started to drop until it started to rise following the injection of the ascaris extract. There was often quickening of the heart during the time the blood pressure was rising. This increase in heart rate we believe was not due directly to the ascaris extract but was a secondary effect due to an adjustment of the heart rate to compensate for the low blood pressure resulting from the injection. Dog 5 was the only one that showed a decrease in heart rate following each injection. The blood pressure

records obtained from this dog showed a reaction to ascaris extract, very similar to the effects produced by electrical stimulation of the peripheral end of the vagi. We thought the extract might be acting on the vagal center and causing a slowing of the heart. To test this idea we cut both vagus nerves and the heart rate increased in the usual manner following vagotomy. Several reactions to the ascaris extract were now obtained and without exception the heart rate decreased and the records resembled those taken before the vagi were cut, thus showing that the slowing of the heart was not caused by impulses carried by the vagus nerves. Considering separately the fifty-nine reactions shown in table 1 the changes occurring in heart rate are so variable, as represented by the high standard deviation, that the chances are about equal for an increase or decrease in heart rate. This conclusion is based on a statistical study using the method of Student (1908) which takes into consideration a pairing of results,—the pairs in this case being the heart rate before as compared to the heart rate after the injection of ascaris extract. According to Love (1924) the arrangement of data in pairs for statistical analysis has an advantage over other methods based on mean average. Since our data could be paired we therefore used Student's method.

The force of the heart was greater after the injection of ascaris extract as judged by palpation. In some cases the impact of the heart against the chest wall was very pronounced.

Plethysmograph records of the intestinal volume were taken on two of the dogs and the splenic volume changes were obtained in one dog. Both the intestinal and splenic volumes decreased during the fall in blood pressure (fig. 1). The spleen seemed to decrease more in volume than did the intestines when the ascaris extract was injected. This comparison, however, is only a general one as we did not determine the mass of tissue contained in the plethysmograph. At times irregularities occurred in the intestinal volume during the fall in blood pressure; these were thought to be due to the active peristalsis produced by the ascaris extract. The plethysmograph records show that the fall in blood pressure cannot be accounted for by vasodilatation in the intestines or spleen.

Effect on respiration. The depth of respiration was in many cases not affected although when a change did occur there was always an increase in depth. With medium doses the change was not great but with large doses the depth was noticeably increased and the respirations were labored. Only one dog (no. 1) showed a noticeable increase in the respiratory rate following the injection of the extract (table 1). In this animal we had constant trouble regulating the ether and this may have altered the results. A careful consideration of the data has shown that the rate of respirations is not noticeably changed by ascaris extract.

We have extended our investigation of the ascaris extract to include ten cats under chloralose anesthesia. The results obtained compare

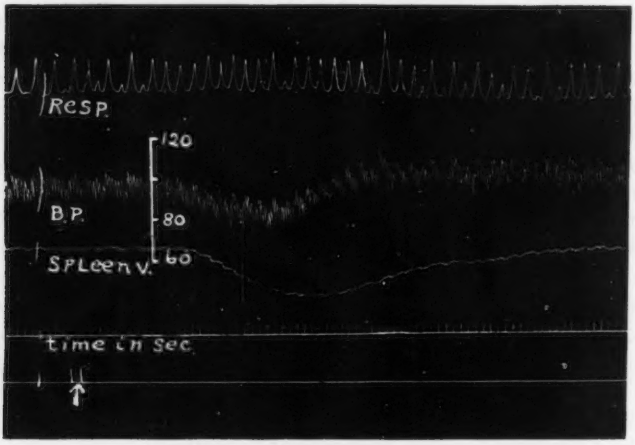

Fig. 1. The effect on respiration, blood pressure, and spleen volume of 0.25 cc. of ascaris extract injected into the femoral vein of a dog under ether anesthesia.

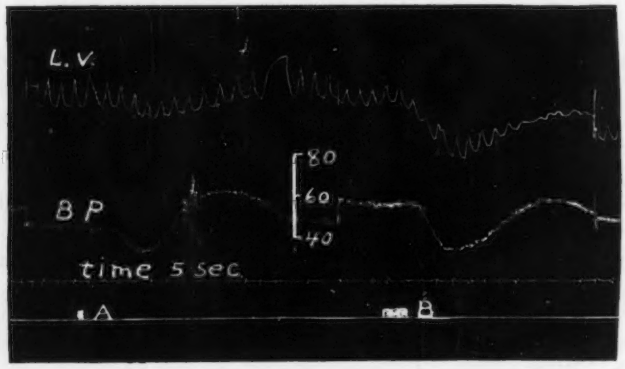

Fig. 2. Liver volume and blood pressure of a cat under chloralose anesthesia. Injections were made into the portal vein of: A, 0.2 mgm. of histamine; B, 0.04 cc. of ascaris extract.

favorably to those obtained from a study of the dogs. We obtained the usual typical fall of blood pressure with either a slight or no change in heart rate. The respirations were, as in the dogs, not noticeably affected.

Plethysmograph records showed a decrease in intestinal volume during the fall of blood pressure confirming our observations on the dogs. In addition we obtained volume changes in the liver and leg. The liver plethysmograph was the type used by one of us (Griffith and Emery, 1929) to obtain vasomotor responses in the liver. The ascaris extract caused a decrease in liver volume almost identical to that brought about by small

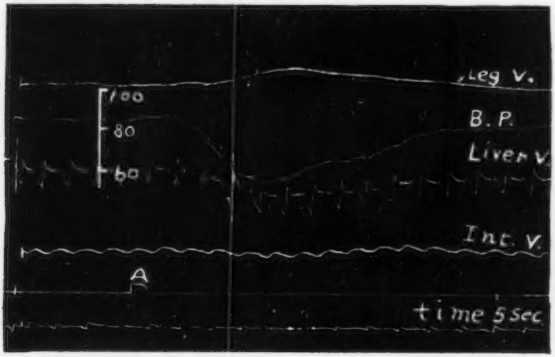

Fig. 3. Blood pressure, leg, intestional, and liver plethysmograph records of a cat under chloralose anesthesia. A, 0.04 cc. ascaris extract injected into the femoral vein. The leg volume is a tracing from another similar record.

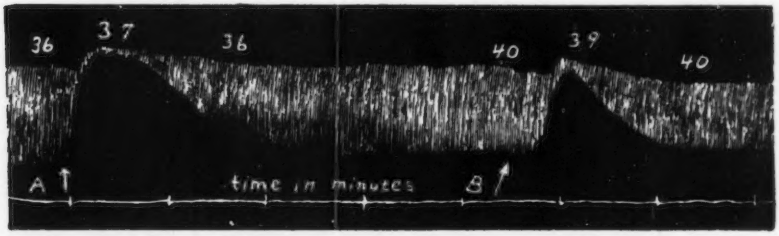

Fig. 4. Record of a turtle's ventricle perfused with Locke's solution. A, 0.1 mgm. of histamine put into the Locke's solution near the heart; B, 0.025 cc. ascaris extract.

amounts of histamine (fig. 2). The leg increased slightly in volume, as shown in figure 3, thus differing from the spleen, intestines and liver which decreased in volume. The rise in the volume of the leg, which was thought to be vasodilatation in the skin, does not seem to be marked enough to account for the fall in blood pressure.

It is interesting to compare the effects produced by histamine and by ascaris extract when applied to the surface or perfused through the heart

of the turtle. Ascaris extract like histamine if concentrated enough to produce a change in the heart rate causes a slowing. This decrease in rate is more easily obtained with ascaris extract than with histamine, but was obtained with either, in normal and in hearts in which the vagal endings were paralyzed with atropine. Although not studied in detail our results on the turtle support the observations of Einis (1913) that histamine causes a slowing of the frog's heart. The most frequent reaction obtained with either histamine or ascaris extract was a calcium like effect with little or no decrease in heart rate (fig. 4).

Discussion. In order to minimize the effect of anesthesia upon the results, the ether was discontinued shortly before the ascaris extract was injected and remained off during the time the changes in blood pressure were being recorded. In a few cases the extract was injected while the dog was under very deep anesthesia and the usual, typical reaction occurred. This led us to regard the influence of the ether as a minor factor in these experiments.

As mentioned above, the ascaris extract contained a tonus increasing substance for the intestinal muscle and contained little if any of the tonus decreasing substance described by Rost (1919). The active substance or substances in the ascaris extract involved in producing the changes described are not known. Our blood pressure records resemble in a marked degree those published a number of years ago by Mott and Halliburton (1899). These investigators were using a brain extract in which the active principle was thought to be choline (Halliburton, 1901). We have not obtained an increase in the intestinal volume with ascaris extract as has been reported for choline (Dale, 1914).

In a previous paper (Herrick and Emery, 1929) we mentioned that the active principle in the ascaris extract did not seem to give entirely a histamine reaction on smooth muscle. We have repeated and confirmed our results that the rabbit's intestine is quite sensitive to ascaris extract. As little as 0.01 cc., containing one or two milligrams of dry material in 100 cc. of Locke's solution, caused a marked increase in the tonus of a segment of the rabbit's intestine. Five milligrams of histamine given under the same conditions as the ascaris extract caused very little change in tonus. This confirms Olivecrona (1921) that the rabbit's intestine is not very sensitive to histamine and also gives further evidence that the active substance in the ascaris extract cannot be histamine only. The depressor action of the ascaris extract is similar to the histamine like action of liver and tissue extract reported by Abel and Kubota (1919) and Burnett (1926) and we were able to obtain a positive histamine reaction (see Koessler and Hanke, 1919) indicating that histamine may have been present. We feel, however, that our data adequately support our conclusion that histamine alone was not responsible for the various reactions obtained.

SUMMARY

1. Changes in circulation and respiration produced by extracts of the pig ascaris have been studied on etherized dogs and cats under chloralose anesthesia.

2. The ascaris extract has a strong depressor effect on blood pressure. The heart rate is not appreciably altered.

3. The respirations were somewhat deeper with little or no change in rate.

4. Plethysmograph records show the volume of the spleen, liver and of the intestine fell with the blood pressure. The volume of the leg increased.

5. The ascaris extract produced irritation to the skin and respiratory passages in man.

6. The histamine like properties of the extract are discussed.

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OBSERVATIONS ON THE RÔLE OF TISSUES IN MAINTAINING THE ACID-BASE EQUILIBRIUM OF THE BLOOD

III. Further Studies on the Hind-Leg Preparation

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In previous studies two of us (Katz and Banus, 1927) demonstrated that during the perfusion of the hind-leg preparation of the dog with blood in which the CO_2 tension was kept constant, the acid-base balance of the blood disturbed by the addition of acid had a tendency to return toward normal. The results indicated that an increase occurred in the amount of base that can combine with CO_2. We concluded from our observations that the muscle tissue was apparently not concerned in this action and that the increase in base was not due to a migration of the chloride ion from the blood to the tissues. The present study was undertaken in part to see if a release of calcium from bone or an outpouring of red blood cells from the bone marrow was responsible for the effect exerted by the tissues in buffering the acidified blood, and in part to reinvestigate the chloride changes. We were not entirely satisfied with our previous chloride results for the following reasons: 1. On recalculating the changes in CO_2 combining power following perfusion with acidified blood we found that a change in chloride of a smaller magnitude than our experimental error of ± 2 millimols could account for the acid-base alteration. 2. The changes in chloride might have been masked by blood dilution or concentration as a result of a continuous exposure to the air-CO_2 mixture in the artificial lung. 3. There was now available the Wilson and Ball (1928) improvement of the Van Slyke method of determining chlorides, which cut down our experimental error to ± 0.3 millimol on the average.

Procedure. The method of experimentation was the same as previously used (1927). In brief, the amputated hind-leg of an etherized dog was connected to the perfusion apparatus and perfused with the defibrinated blood of another dog. During the entire course of the experiment the CO_2 tension of the air-CO_2 mixture was maintained constant at 30 mm. Hg. After approximately $\frac{1}{2}$ to $\frac{3}{4}$ of an hour, a control sample was taken for

analysis (sample I). Immediately thereafter 20 per cent by volume of a solution of N/12 HCl dissolved in isotonic saline was slowly added and mixed for 10 minutes through a shunt. A second sample of blood was then drawn (sample II) to determine the effect of the acid solution on the blood. Perfusion through the hind-leg was then continued for one hour after which a third sample (sample III) was taken. The difference between these last two samples indicates the effect exerted by the tissues of the hind-leg on the acidified blood under these conditions.

The pH determinations were carried out in the manner previously described in experiments 46 and 47 only. These determinatons were discontinued as the results agreed with those obtained previously and they were not needed to calculate the millimol changes of base in the blood. The CO_2 combining power was measured as before except that in the present research the equilibration of the blood sample was done at 38°C. with a CO_2 tension of 30 mm. Hg, the same tension as existed in the lung of the perfusion system. The experimental error with this method was ± 0.25 volume per cent (0.11 millimol), a maximum of ± 0.5 volume per cent. The chlorides of whole blood were measured, as indicated above, by the Wilson and Ball modification (1928) of the Van Slyke method, a modification which reduced our experimental error to an average of ± 0.3 millimol (a maximum error in one case of ± 1.8). The calcium in whole blood was measured by several different methods. The Rothwell method (1927), which we felt gave our best results, had in our hands an experimental error averaging ± 0.06 mgm./100 cc. (a maximum error of ± 0.20). We also checked our calcium values with the Roe and Kahn technique (1929), our experimental error with this method was ± 0.19 mgm./100 cc. In the first few experiments the Clark method (1921) was used instead of the Rothwell, the experimental error with this procedure was ± 0.29 mgm./100 cc. We found close agreement between the Rothwell and the Roe and Kahn, although the values with the latter ran 0.45 mgm./100 cc. higher than those with the former. The values found with the Clark method ran 1.07 mgm./100 cc. lower than those simultaneously obtained with the Roe and Kahn. The data given in tables 1, 2 are based on the Rothwell method except in experiments 46 and 47, in which the values with the Clark method are given. Since it was not possible to keep the air-CO_2 mixture in the lung of the perfusion apparatus exactly just saturated with moisture—although this condition was approximated even better than in previous experiments—account was taken of changes in blood concentration in calculating the results. At times the air-CO_2 mixture was partially saturated leading to evaporation of water from the blood and at other times it was supersaturated, causing a blood dilution. The change in water content of the blood was determined gravimetrically, except in experiments 46 and 47, according to Van Slyke's method (1923). Our experimental error with this method was ± 0.1

TABLE 1

A. Effect of hind-leg preparation on the acid-base equilibrium, chloride and calcium content of blood acidified with hydrochloric acid

Sample I—control
Sample II—after acid added and 10 minutes' mixing
Sample III—1 hour after sample II

EXPERIMENT NUMBER	SAMPLE NUMBER	BLOOD FLOW	BLOOD VOLUME	CHLORIDES AS NaCl	CO_2 COMBINING POWER	pH	Ca	CONCENTRATION OF BLOOD	R.B.C. COUNT
		cc. per minute	*cc.*	*mgm. per cc.*	*volumes per cent*		*mgm. per 100 cc.*	*per cent of water*	*per cubic millimeter*
47	I		550	6.08		7.05	7.63		
	II		580	6.94		6.72	6.46		
	III		550	6.78		6.82	7.11		
48	I	23	600	5.28	27.9		6.90	80.1	
	II		530	5.80	17.5		6.45	82.2	
	III	46	400	5.74	20.1		6.60	81.2	
				(5.66)	(19.9)		(6.51)	−1.23%	
49	I	43	650	4.36	5.7		6.93	80.8	2,976,000
	II		450	6.47	3.0		6.62	83.2	2,428,000
	III	190		6.36	4.7		6.71	83.2	2,148,000
								±0.00%	
50	I	33	550	4.81	26.4		6.99	80.5	5,172,000
	II	63	550	6.07	14.9		6.46	83.0	3,648,000
	III	57		5.94	17.9		6.77	83.5	2,984,000
				(5.97)	(18.0)		(6.83)	+0.66%	(3,010,000)
53	I		600	4.94	26.0		6.13	79.6	
	II	40	640	6.13	13.1		5.91	82.1	
	III	70	500	6.03	15.8		5.89	82.0	
				(6.02)	(15.7)		(5.88)	−0.12%	
54	I	15	700	4.89	23.8		5.42	78.1	
	II	37	550	5.94	15.6		5.09	80.3	
	III	30	350	5.90	17.4		5.35	80.3	
								±0.00%	
55	I	36	600	5.07	25.7		7.07	79.0	
	II	53	650	6.20	17.0		6.49	81.7	
	III	65	500	6.08	19.4		6.81	81.7	
								±0.00%	
56	I		700	4.84	23.0		6.11	78.2	
	II	35	550	6.11	12.1		5.98	81.2	
	III	55	500	5.87	14.8		6.34	80.7	
				(5.83)	(14.7)		(6.29)	−0.62%	

TABLE 1—*Concluded*

B. Controls

Sample I—control
Sample II—after NaCl solution added and 10 minutes' mixing
Sample III—1 hour after sample II

EXPERIMENT NUMBER	SAMPLE NUMBER	BLOOD FLOW	BLOOD VOLUME	CHLORIDES AS NaCl	CO_2 COMBINING POWER	pH	Ca	CONCENTRATION OF BLOOD
		cc. per minute	*cc.*	*mgm. per cc.*	*volumes per cent*		*mgm. per 100 cc.*	*per cent of water*
46	I			6.24		7.20	7.24	
	II*			6.15		7.19	7.15	
	III			6.23		7.19	7.19	
52	I	28	550	5.01	21.0		5.84	78.9
	II	27	540	5.28	21.2		5.55	81.2
	III			5.30	20.3		5.78	81.5
				(5.28)	(20.2)		(5.76)	+0.33%
57	I	30	650	5.32	22.7		7.12	70.5
	II	41	625	5.93	21.6		6.75	82.6
	III	55		5.87	21.3		7.12	83.0
				(5.84)	(21.2)		(7.09)	+0.49%

* No NaCl solution added.

per cent of water. Red blood cell counts were made in two experiments with a hemocytometer.

Control experiments were made to eliminate the effect of perfusion for the same period of time. In two of the control experiments the technique of the acid experiment was further approached by adding 20 per cent by volume of an isotonic NaCl solution to the blood after taking the first sample, and mixing for 10 minutes before taking the second sample of blood.

RESULTS. The data of the eight experiments performed are given in table 1. Attention is directed to the data in samples II and III in each experiment which gives the information concerning the interchanges between the tissues and the acidified blood of the perfusate. The figures given in parenthesis with sample III are the values corrected for the change in water content of the blood caused by exposure to the gas mixture in the artificial lung; a variation ranging from a loss of 1.2 per cent in experiment 48 to a gain of 0.66 per cent in experiment 50 was found. The differences in each experiment between sample II and the corrected value of sample III are assembled, together with their equivalents in millimols/liter in table 2-A.

In all instances, as examination of tables 1-A and 2-A shows, perfusion of acidified blood through the hind-leg leads to a return of the acid-base balance back toward normal, as indicated by the increase in pH in experi-

ment 47 and by the increase in CO_2 combining power in the other experiments. These changes are of the same order of magnitude as in our previous experiments.

The red blood cell counts in experiments 49 and 50 show that the increase in CO_2 combining power is not caused by an outpouring of red cells from the bone marrow. In both cases, in fact, the count decreased, probably due to a slight hemolysis.

TABLE 2

Changes in calcium, chlorides and CO_2 combining power of acidified blood perfused for 1 hour through the hind-leg preparation

EXPERIMENT NUMBER	Cl		CO_2 COMBINING POWER		Ca		pH
	Milligrams per cc. as NaCl	Millimols per liter	Volume per cent	Millimols per liter	Milligrams per 100 cc.	Millimols per liter	
Recovery from acid (A)							
47	−0.16	−2.76			+0.65	+0.16	+0.10
48	−0.14	−2.33	+2.4	+1.05	+0.06	+0.015	
49	−0.11	−1.90	+1.7	+0.76	+0.09	+0.023	
50	−0.10	−1.73	+3.1	−1.38	+0.37	+0.09	
53	−0.11	−1.90	+2.6	+1.16	−0.03	−0.008	
54	−0.04	−0.52	+1.8	+0.82	+0.26	+0.07	
55	−0.12	−2.09	+2.4	+1.05	+0.32	+0.08	
56	−0.28	−4.83	+2.6	+1.15	+0.31	+0.08	
Average	−0.13	−2.24	+2.3	+1.00	+0.25	+0.06	
Controls (B)							
52	±0.00	±0.00	−1.0	−0.40	+0.21	+0.05	
57	−0.09	−1.45	−0.40	−0.16	+0.34	+0.085	
Average	−0.045	−0.72	−0.70	−0.28	+0.275	+0.07	
Difference between average A and average B		−1.52		+1.28		+0.01	

With the exception of experiment 53[1] an increase occurred in the calcium in the blood, but the change was small, less than $\frac{1}{10}$ of the change in buffering power. Apparently calcium liberation does not account for the increase in CO_2 combining power. The slight increase in calcium is no greater in the acid experiments than the change in the controls (cf. tables 1-B and 2-B). We are unable to say whether the increase in calcium in both

[1] In this experiment an insignificant decrease was found. The Roe and Kahn method, however, showed an increase of 0.31 mgm./100 cc.

control and acid experiments is due to the liberation from the bone or from some part of the rubber-glass perfusing system.

Contrary to our previous results, we found that the migration of chlorides to the tissues is sufficient to explain the increase in CO_2 combining power. In every experiment of this series, the chlorides in the blood decrease after perfusion through the hind-leg preparation, and the decrease in chloride in each case is of the same order of magnitude as the increase in CO_2 combining power (cf. table 2-A). This shift in chlorides was masked by the greater magnitude of our experimental error in the previous experiments. It is significant, although we do not lay too much stress on the point, that the difference between the average values of the acid and control experiment for the chloride change is approximately the same as that for the CO_2 combining power change (when both are expressed in millimols/liter (cf. table 2)).

Discussion. Our results indicate that the increase in CO_2 combining power and the restoration of pH toward normal can be explained by a shift in chlorides from blood to tissues. Chlorides can therefore penetrate into some of the tissues[2] present in the hind-leg preparation as well as into the red blood cell. In the intact animal a transportation of CO_2 probably occurs, but our experiments were designed especially to e liminate this possibility, in order to establish whether or not any further buffering action of the tissues was possible.

Our results further indicate that there is no outpouring of red blood cells from bone marrow when the marrow is exposed to acidified blood. Recently, Myers, Muntwyler and Pomerene have found an increase in red cells in blood during anaesthesia acidosis which was not due to blood concentration and which, in view of our findings, would probably indicate an outpouring from other organs such as the spleen.

Finally our results indicate that perfusion of acidified blood through bone does not cause the liberation of large amounts of calcium. No difference was found in the amount of calcium which appears in the blood, regardless of whether acidified or normal blood was used in perfusion. A controversy has recently arisen in regard to the rôle of calcium in buffering acid in the blood. An excellent review of this subject is given by Stewart and Percival (1928). The results of Allers and Bondi (1907) and Stewart and Haldane (1924) indicate that blood calcium content and urine calcium excretion both increase in conditions of acidosis. Givens (1918) and Lamb and Evvard (1919) maintain that the rôle in buffering played by the calcium increase in blood and urine is small, whereas Cantarow, Caven and Gordon (1926) believe the effect is nil. As far as our results indicate anything,

[2] Other than muscle. Our previous results on the lack of chloride shift into muscle was confirmed by Mond (Pflüger's Arch. 1928, ccxx, 69).

they favor the view that calcium in bone is not available to any great extent as a source of buffer material for acidified blood.

SUMMARY

1. The finding is confirmed that the perfusion of blood acidified with hydrochloric acid through a hind-leg preparation of a dog tends to restore the acid-base equilibrium of the blood toward normal.

2. It is shown that the increase in CO_2 combining power (and pH) of the blood may be explained by a migration of chloride from the blood to some tissues in the hind-leg preparation.

3. Perfusing acidified blood through bone tissue causes no outpouring of red blood cells from its marrow, nor any significant liberation of calcium under the conditions of the experiments.

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A NOTE UPON LYMPH FORMATION IN THE DOG'S KIDNEY AND THE EFFECT OF CERTAIN DIURETICS UPON IT

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The possible significance of lymph formation and lymph flow in the kidney in relation to the processes of urine formation has received comparatively little attention. The possibility of lymph formation within the kidney was shown by Ludwig and Zawarykin (1) when they obstructed the ureter and found that lymph vessels leaving the kidney at its hilus became distended; anatomical investigations (2) (3) (4) have demonstrated the presence of a well-developed lymphatic system in the kidney, but evidence bearing upon the physiological importance of this system appears to be lacking. Heidenhain (5) states that Ludwig, as a result of the above-mentioned investigation (1), came to regard lymph in the peritubular spaces as the fluid concerned with tubular reabsorption.

Our attention was drawn to this question during the course of an investigation of the gaseous metabolism of the dog's kidney (6). It was occasionally noted that renal venous blood, collected during profuse diuresis, was more viscid than arterial blood, and in one case renal venous blood actually had a higher oxygen content than arterial. Such differences were naturally attributed to loss of fluid as urine. However, when the loss of fluid necessary to account for the observed concentration of renal venous blood was calculated from the oxygen capacities or hematocrit readings of the blood samples, or from refractometric estimations or dried weights of serum, the value was usually greater than the observed volume of urine. No such discrepancy was evident when diuresis was absent.

Thus, in one experiment during sulphate diuresis:

O_2 capacity of arterial blood	15.73 vol. per cent
O_2 capacity of renal venous blood	16.31 per cent
Renal blood-flow per minute	214 cc.
Calculated loss of fluid from blood per minute	7.62 cc.
Measured rate of urine flow per minute	4.4 cc.
Calculated loss of fluid unaccounted for per minute	3.22 cc.

In another experiment during sulphate diuresis:

Cell volume of arterial blood (hematocrit)	33.6
Cell volume of renal venous blood	34.2
Renal blood-flow per minute	40 cc.
Calculated loss of fluid per minute	0.704 cc.
Measured rate of urine flow per minute	0.390 cc.
Calculated loss of fluid unaccounted for per minute	0.314 cc.

Four other experiments yielded similar results.

While the accuracy of the methods employed cannot be completely guaranteed, the consistency of the results gave strong indication that fluid left the blood in its passage through the kidney by some other route than that of urine. Accordingly we investigated the influence of diuretics upon lymph flow, and the contribution which the kidney might make to the stream collected from accessible lymph vessels, under the conditions of our experiments. This incomplete report is now presented because our further collaboration must be postponed indefinitely.

Nine experiments were made upon dogs narcotized with luminal and prepared for measurement of blood-flow through the left kidney according to the Barcroft-Brodie method. The gastro-intestinal tract and right kidney were removed, the portal vein and hepatic artery were tied, and the aorta and inferior cava were ligated just below the level of the left renal vessels. The left kidney was therefore the only abdominal viscus remaining to contribute to total lymph flow. In four experiments a cannula was inserted into a tributary of the abdominal lymph receptaculum just below the kidney; in the others this was not possible and collection was made from the thoracic duct in the neck.

In these experiments diuretic agents uniformly increased outflow of lymph, at the same time that urine flow and renal blood-flow were increased. Such agents included caffeine and theocine, and hypertonic solutions of sodium chloride, sulphate, and phosphate, given by intravenous injection. Adrenalin, on the other hand, also increased lymph flow while decreasing urine and renal blood flow. Data taken from the records of three experiments which are representative of results consistently obtained are as follows:

	Urine flow	Lymph flow
	(*cc. per minute*)	
I. At beginning of experiment	0	0.04
During sulphate diuresis	0.25	0.20
After phosphate	2.36	0.60
Recovery	1.3	0.23
After adrenalin	0.81	0.32

II. At beginning of experiment	0.36	0.03
After caffeine	0.56	0.11
III. At beginning of experiment	0.04	0.15
After sodium chloride	0.13	0.43

In order to determine whether or not the kidney had contributed to the observed lymph flow, two sorts of procedures were employed: first, occlusion of the renal artery or vein in vivo, observing the effects upon lymph flow; second, perfusion of the renal blood vessels immediately after death of the animal, noting effects upon outflow from the lymph cannula. The results of both types of experiment indicated that the kidney was capable of contributing a very considerable share to the observed lymph flow.

Two representative examples of the effects of obstruction of renal artery and vein upon total lymph flow in the living animal with active kidney are as follows:

	Urine	Lymph
	(cc. per minute)	
I. Control period (sulphate diuresis)	1.5	0.3
Occlusion of one branch of renal artery	0.25	0.17
Recovery (plus phosphate)	2.1	0.17
Occlusion of renal vein	0.6	0.44
Recovery	1.1	0.12
II. Control period	0.08	0.06
Occlusion of renal vein	0.0	0.26

In the perfusion experiments the results were more striking. In one selected case, perfusion of the renal artery with oxygenated Locke's solution led to an escape of approximately 200 drops (12.5 cc.) of fluid per minute from the ureter, while 6 drops (0.33 cc.) per minute issued from the cannula in the thoracic duct; upon transferring the perfusion stream to the renal vein, only one drop of fluid per minute escaped from the ureter, while lymph flow was exactly doubled (0.66 cc. per minute). In this, as well as in the other perfusion experiments, the animal's heart had stopped and there was no flow from the lymph cannula before perfusion was begun; interruption of the perfusion caused gradual cessation of flow from the lymph cannula.

Additional information was obtained from injection experiments. A diffusible dye (methylene blue) was added to the Locke solution perfused via the renal artery in four experiments: in two, it appeared promptly in the "urine," and, several minutes later, in the fluid collected from the thoracic duct; in the other two there was no flow of "lymph" during the perfusion. India ink was added to the arterial perfusion fluid in four other experiments; it did not appear in either "urine" or "lymph," though both

continued to flow freely. These results indicate that in these animals there were no direct communications between the blood and the lymph vascular systems in the kidney. A terminal injection of india ink was made into the lymph cannula in nearly every experiment: even when fairly high pressure was used there was no escape of the stain into the abdominal cavity, showing that the outflow from the lymph cannula had actually come from lymph spaces.

These results indicate that fluid can leave the blood within the kidney to enter lymphatics as well as ureter, and that escape through both channels is augmented by the same agents, i.e., by diuretics.

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THE EFFECT OF SECTION OF THE VAGUS NERVES ON GASTRIC ACIDITY

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Much experimental work has been done with regard to the relation of the autonomic nervous system to the stomach. Clinical and experimental observers have reported lowering of gastric acidity following section of the vagus nerves.[1] These observations have led to the assumption that the cutting of the vagus nerve supply to the stomach might be of definite value in the treatment of certain functional and organic diseases in human beings and by others who have worked with experimental animals. The latter have observed, further, a beneficial effect of section of the vagus nerves on hypertonicity and spasm. Others believe these beneficial results are vitiated because of gastric atony, dilatation and ulcer.

Clinical observations have been very incomplete. Moreover, the results of section of the vagus nerves on the experimental animal have been largely inconclusive because: 1, the preoperative normal values have not been adequately determined; 2, operations for the production of fistulas and pouches had been performed previously, and 3, the period of postoperative observation has been too brief.

This experimental study is concerned only with the effect of section of the vagus nerves on the acidity of the gastric content. The normal acidity was well established. The observations were made on animals on which operation other than section of the vagus nerves was not performed. The degree of acidity was observed postoperatively over long periods of time.

HISTORICAL DATA. The study of the effect of section of the vagus nerves on gastric acidity goes back over a hundred years. One of the earliest investigators was Brodie (1814), who observed that the stomachs of dogs killed by intravenous injections of arsenic contained copious secretions of mucus and watery fluid. If, however, the vagus nerves were severed previous to the administration of a lethal dose of arsenic the stomachs were always empty. These observations were confirmed by Wood (1870) with

[1] The expression, section of the vagus nerves, unless otherwise designated refers to the cutting of the vagus nerve supply to the stomach.

the further observation of failure to produce purgation following section of the vagus nerves. Bidder and Schmidt (1852), Schiff (1860), Vulpian (1875), Bernard (1876), Richet (1878), Regnard and Loye (1885), Axenfeld (1890), Countejean (1896), Krehl (1892), and Cheshkoff (1900 and 1902), while experimenting along different lines, came to the conclusion that the vagus nerves play an important part in regulating the gastric secretion. Leubuscher and Schäfer (1894), Boruttan (1896), and Herzen (1897), experimenting on dogs, observed failure in gastric digestion following section of the vagus nerves. The latter obtained normal digestion by introducing hydrochloric acid into the stomach through a gastric fistula. Litthauer (1920) observed this inability of the stomach to digest food following vagotomy. Pavlov (1894) made a similar observation on dogs and also found that hydrochloric acid was never present following section of the vagus nerves, but that it could be stimulated by the introduction of bouillon, and the addition of normal gastric juice from other dogs was found to be a marked aid to digestion. He confirmed his observation over a period of six months (1895–1896). Pavlov's classic experiment, reported later (1910), proved that the vagus nerves supply secretory fibers to the gastric glands, and that they are the pathway for the stimuli which excite the psychic phase of gastric secretion. He also showed that these psychic stimuli will cause a constant and profuse flow of gastric juice for several hours or as long as the animal is sham-fed, and also that on removal of the animal's food the secretion does not stop immediately but "continues for a long time, three to four hours, gradually dying out." Ivy (1925), Lim, Ivy, and McCarthy (1925), and Farrell (1928) have made valuable contributions to this subject, and in general confirm the work of Pavlov.

In the course of the last fifteen years surgeons have been cutting the vagus nerves in the abdominal cavity in human beings. Exner and Schwarzmann (1914) reported twenty cases of gastric crises in which vagotomy was performed with beneficial results in 50 per cent of the cases. Ducceschi (1917), Bircher (1920), Stierlin (1920), and Latarjet (1922) all experimented on dogs and came to the general conclusion that section of the vagus nerves definitely lessened the secretory activities of the stomach. They have performed various types of denervation of the stomach on human beings, in which the nerve fibers cut were predominantly those of the vagus nerves, for the relief of hyperacidity and hypersecretion, and hypertonicity and spasm. They believe that beneficial results were obtained. Borchers (1921) disagreed with Bircher and Stierlin with regard to any good effect which section of the vagus nerves might have on disturbance in motility, but believed that such an operation might be useful solely for the relief of hypersecretion and hyperacidity. However, Borchers did not mention cutting the nerve supply to the region of the sphincter. McCrea (1924) has shown that the nerve supply of this region

is derived from the hepatic branch of the vagus nerve and therefore would not be cut in the type of denervation performed by Borchers. Latarjet's operation is very accurate, and the technic is described in detail. Hughson (1927), McCrea, McSwiney and Stopford (1926) in experimental studies on dogs reported that the pylorus tends to be patulous following section of the pyloroduodenal nerves described by McCrea. Latarjet reported twenty-four cases in which the patients were benefited clinically from his operation. He advised the operation in gastric crises, but especially in those forms of gastropathy in which there are signs of functional disequilibrium but in which there are no organic lesions. In cases of gastric and duodenal ulcer he believes denervation is indicated and when stenosis and pyloric obstruction are present this operation may be combined with gastro-enterostomy or pylorectomy. Pauchet (1923), following the method of Latarjet, performed neurectomy in twelve cases, with beneficial results. Gianolla (1923) and Schiassi (1925) sectioned the branches of the vagus nerves in the abdomen of human beings in cases of hyperacidity and hypertonicity, with beneficial results. In cases of gastric and duodenal ulcer, Gianolla advised resection of the lesion, supplemented by section of the nerve. Schiassi believes that denervation is sufficient in simple duodenal ulcer but in the presence of hemorrhage or stenosis he performs gastro-enterostomy also. He has operated in twenty-five cases, with favorable results. C. H. Mayo (1928) advised section of the branches of the vagus nerves to the pylorus for the relief of spasm. He severs nerves along the lesser curvature on the anterior and posterior walls of the stomach above the angle. He also divides the pyloroduodenal branch which descends from the liver.

Foa (1927), Dudek (1927), and Alvarez (1928) reported lowering of gastric acidity following experimental vagotomy.

Method of experiments. The animals used were carefully selected, healthy, adult dogs of medium weight and in good flesh, ranging in weight from 10 to 18 kgm. They were allowed to become familiar with their surroundings and soon became friendly. Fractional gastric analyses then were performed after the method of McCann. Following a fast of eighteen hours the animals were fed a test-meal consisting of 80 grams of fresh, lean, finely ground horse meat and 250 cc. of water. Small samples of the gastric contents were then removed by means of a Smithies stomach tube inserted through the esophagus in the same manner as that employed in performing gastric lavage in the human being except that the jaws are held apart by a small wooden gag which has a hole in the center to permit the passage of the tube. The first sample was removed half an hour after the test-meal had been given, and aspirations were made every half hour thereafter until free hydrochloric acid appeared. They were then made every fifteen minutes until free hydrochloric acid was no longer recovered. Each

sample was centrifugalized and the juice was titrated against N/10 sodium hydroxide. The content of free hydrochloric acid was determined by means of Töpfer's reagent (dimethylaminoazobenzene) and the total acid by means of phenolphthalein. The figures recorded indicate the number of cubic centimeters of N/10 hydrochloric acid and of N/10 total acids in 100 cc. of gastric content. The hydrogen-ion concentration of each sample was determined at room temperature by means of a Leeds-Northrup potentiometer.

The aspirations were carried out two to three times a week on each dog, until each animal had become accustomed to the procedure. The length of time required for training each dog varied from one week to six weeks. Other animals were substituted for animals that were found not to be suit-

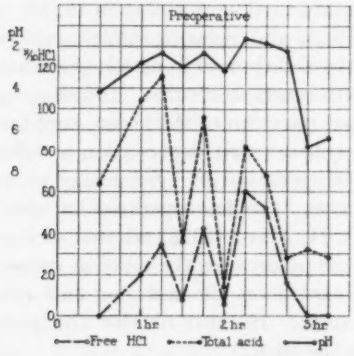

Fig. 1. The effect of a psychic upset on the acidity of the gastric juice. This animal was in two short fights at about one hour and twenty minutes and one hour and fifty minutes after feeding time, respectively.

able. During the period of training the acidity of the gastric content varied greatly, especially the amount of free hydrochloric acid. It was apparent that any psychic upset, no matter how slight, would cause a lessening of the hydrochloric acid which, depending on the degree of the psychic disturbance, might disappear altogether. There would be a corresponding diminution in total acidity and an increase in the value for hydrogen-ion concentration. If the animals were reprimanded or got into a fight at a time when the hydrochloric acid was being secreted the curve of acidity would drop immediately, and would rise again in fifteen to thirty minutes. In the beginning, such a fluctuation often was caused by intubation (fig. 1). During the period of training, each animal's stomach was aspirated following an eighteen-hour fast and before the administration of the test-meal; retention never was present. Occasionally during the course of

aspiration a piece of gastric mucosa would be sucked up, and blood would appear in the specimen. This would cause a rise in the acidity (fig. 2).

After the animal was well trained, the acidity began to show a fairly constant value. Five to eight fractional analyses then were made over a period of three to five weeks, and graphs were made of each, showing the amount of N/10 free hydrochloric acid present, the total acidity and the hydrogen-ion concentration of the gastric content from half an hour after ingestion of the meal until after the stomach was empty and the free hydrochloric acid had disappeared.

In all, eight animals were used and two types of operations were performed. The vagus nerves of six dogs were cut intrathoracically following the method advocated by Mann. Briefly the technic was as follows: The animals were prepared by a twelve-hour fast. Ether was administered intratracheally by means of a Connel artificial respiration machine. Aseptic technic was used. The chest was opened in the seventh or in the eighth intercostal space on the left side. The lower lobe of the left lung was packed off. The anterior and posterior vagal trunks were then exposed just above the diaphragm as they lay just beneath the pleura, one anterior and one posterior to the esophagus. These were isolated, picked up, and a small piece, about 0.5 cm. long, was removed from each. The chest was then closed. The ribs on either side of the incision were approximated by interrupted linen sutures. The muscles and fascia were closed in layers.

In two dogs, under ether anesthesia administered intratracheally and with aseptic technic, the branches of the vagus nerves in the abdomen were cut. The stomach was exposed through a median-line incision. Traction was then made on the fundus of the stomach, so that the region of the lesser curvature, just above the angle, was brought into view. An incision was then made through the nerves and vessels lying in the gastrohepatic omentum and along the lesser curvature, above the angle of the stomach. An incision, penetrating to the mucous membrane, was carried transversely around the stomach from the lesser to the greater curvature. The posterior wall was approached through openings made in the gastrohepatic and gastrocolic omentum. The serosa was then approximated with fine continuous catgut sutures. Next, care was taken to cut the branches of the pyloroduodenal nerve descending from the hepatic branch to supply the region of the pyloric sphincter. As few vessels as possible were injured.

Food and water were withheld for the first five days after operation, but during this period 150 cc. of glucose, 10 per cent, in a solution of sodium chloride, 1 per cent, were administered intravenously twice a day. On the sixth day the animals were given water, on the seventh milk and syrup, and by the beginning of the second week they were back on their normal diet of biscuit, milk and syrup, and meat. The first fractional

analyses were made on the tenth day and were continued thereafter one to two times a week for one to five months. More than 1000 gastric analyses were made, besides those performed during the period of training.

RESULTS OF EXPERIMENTS. Four of the six dogs in which the vagus nerves were sectioned in the chest recovered uneventfully and have been observed for long periods. Fractional gastric analyses were made during the course of one to five months. All have shown marked and constant reduction in both free hydrochloric acid and total acidity, with a corresponding rise in the hydrogen-ion concentration (figs. 3, 4, 5, and 6). The amount of free hydrochloric acid and total acidity, and the hydrogen-ion concentration have been plotted for each gastric analysis and graphs have been made. It is interesting to note that the highest curve noted postoperatively is lower than the lowest curve obtained previous to section of the vagus nerves. Of these four animals, three are still alive and well, and thus far the secretory curve appears to be unchanged. The other animal died suddenly on the thirty-fifth day following operation, after a brief period of intercurrent pneumonia. He had been well nearly a month and his secretory function had been well studied. Postmortem examination revealed that the vagus nerves had been properly cut. The stomach appeared entirely normal; retention, dilatation, or lesions of the mucosa were not present. Evidence of gangrenous pneumonia was found.

One dog died on the seventh day following operation of an infected aspirating wound and empyema. Postmortem examination showed the nerves to have been sectioned properly. The stomach appeared normal; dilatation, retention, or lesions of the mucosa were not present. The remaining dog made a good recovery following operation; however, the

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Fig. 2. Effect of trauma and the presence of blood on the curve of acidity of gastric content. The trauma took place four hours after feeding. Blood appeared in the specimen obtained at the time trauma occurred and in the next specimen.

Fig. 3 (first dog). Effect on the acidity of gastric content of section of the vagus nerves in the thorax.

Fig. 4 (second dog). Effect on the acidity of gastric content of section of the vagus nerves in the thorax.

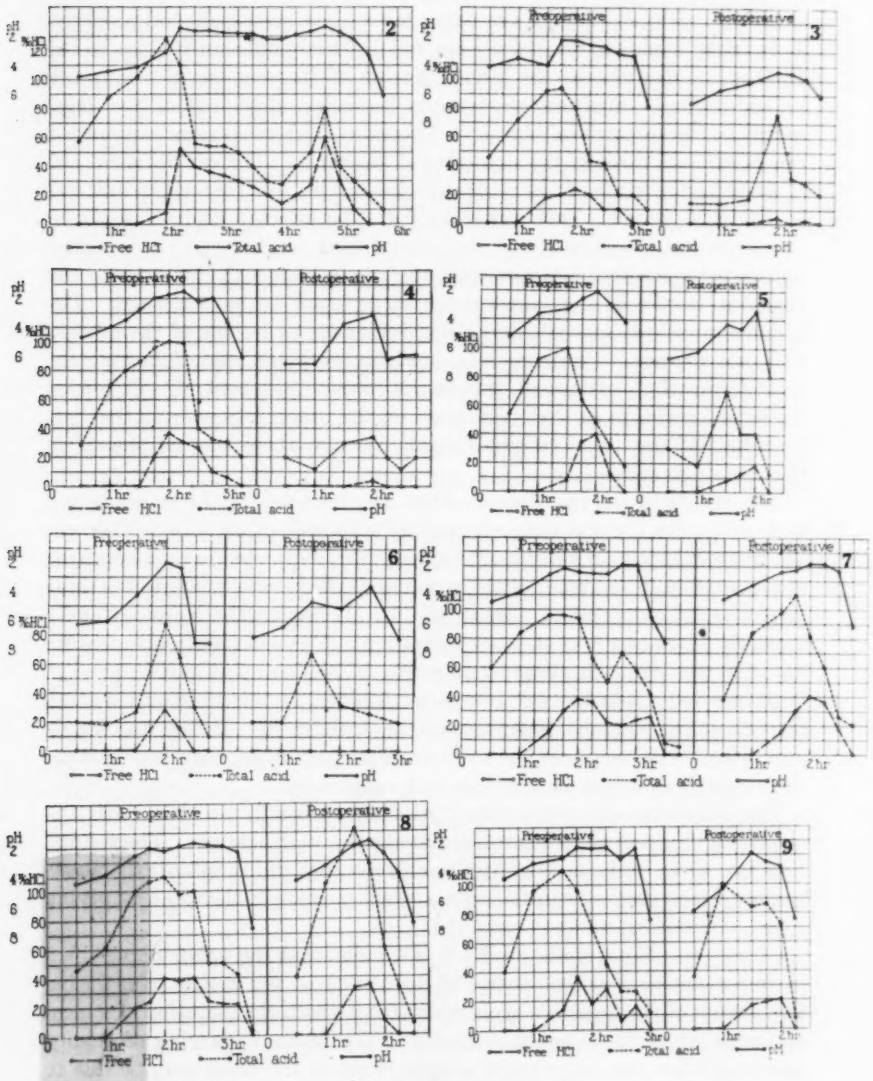
Fig. 5 (third dog). Effect on the acidity of gastric content of section of the vagus nerves in the thorax.

Fig. 6 (fourth dog). Effect on the acidity of gastric content of section of the vagus nerves in the thorax.

Fig. 7 (fifth dog). Effect on the acidity of gastric content of section of the anterior vagus nerve in the thorax. The attempt was to sever both vagus nerves but the posterior trunk was missed. The curves made after operation are essentially the same as those made before operation.

Fig. 8 (sixth dog). Effect on the acidity of gastric content of section of the vagus nerves in the abdomen.

Fig 9 (seventh dog). Effect on the acidity of gastric content of section of the vagus nerves in the abdomen.


2
3
4
5
6
7
8
9
Preoperative
Postoperative
Free HCl
Total acid
pH

Figs. 2 to 9

results of postoperative analyses of gastric contents appeared the same in every respect to those made before operation (fig. 7). The question arose as to whether the trunks of the vagus nerves had been sectioned properly. It is fairly likely, if the trunks are not isolated sufficiently close to the diaphragm, that one of the large branches of the esophageal plexus will be cut instead. Such an error would leave less than half of the vagal supply to the stomach still intact. Therefore, four weeks later exploration was performed through the right side of the chest. The cut proximal end of the anterior trunk of the nerve was located but the posterior trunk was found to be intact. This was sectioned. An infected aspirating wound and empyema developed, and the animal died on the seventh day after the second operation. At postmortem examination both the anterior and posterior trunks of the vagus nerve were found to be sectioned. The stomach appeared normal; dilatation, retention, or lesions of the mucosa were not present. Unfortunately, analyses were not made following the second operation. However, this case is valuable as a control. Following the same operation as that performed on the other dogs, except that less than half of the vagal supply remained intact, there was no change in the secretory curve.

The results in the two dogs in which the branches of the vagus nerves were severed in the abdomen run parallel with each other. In neither was the hydrochloric acid constantly reduced; the hydrochloric acid appeared at the same time and rose a little more rapidly but occasionally to the same height as before operation (figs. 8 and 9). However, the secretory curve in nearly every case was of shorter duration. The most noticeable feature was that the stomach always was empty of all food and gastric juice in from one and a half to two hours after feeding; at this time only a small amount of bile-stained mucus could be withdrawn. Before the vagus nerves had been sectioned, at least one hour more had been required for the stomach to attain such a degree of evacuation.

The stomachs also were examined fluoroscopically to determine the emptying time and also the presence or absence of dilatation. The results, thus far, agree with those of McCrea, McSwiney and Stopford; there was a marked increase in the initial, and a slight increase in the total, emptying time. Dilatation was not observed.

COMMENT. From these results it is evident that the vagus nerves play an important part in the control of the acidity of the gastric juice during that period of the digestive cycle which begins with the ingestion of a meal and ends when the stomach is empty. The marked lowering of acidity observed when the entire vagal supply is cut in the chest may be due to several factors.

1. The psychic phase of secretion is permanently and entirely abolished. This has been proved by Pavlov (1910), and has been confirmed by Farrell

and others. It is difficult to estimate the exact significance of this phase in regard to the total amount of acid and the degree of acidity in the course of digestion. That it is of relatively great importance in the dog is proved by Pavlov's experiment (1910), in which he has shown that the psychic secretion, when once started, continues to flow for two to three hours after the stimuli have been removed. This may vary somewhat in different dogs and with the degree of hunger. However, in well-fed and well-nourished kennel dogs it should not be abnormally great.

2. To what extent the acidity is lowered as a result of some effect on the gastric phase of secretion is conjectural. If the gastric phase of secretion is in part dependent on stimulation of the vagus nerves, as is believed by Polkras and Michelson, section of the vagus nerves should lower both the quantity and acidity of the gastric juice. On the other hand, if the gastric phase of secretion is dependent on the absorption of partially digested food, such as histamine and the juice of raw meat, as is believed by Lim, Ivy, and McCarthy, the severance of the vagus nerves will delay the digestion to such an extent that partial evacuation will take place before this absorption can occur. Thereby, the gastric phase of secretion will be shortened to such an extent that the normal concentration of acid is never reached.

3. The intestinal phase of secretion may be affected in a like manner, for, as has been shown by Ivy, Lim, and McCarthy, this is dependent on the action of digested complex food substances, such as peptones and amino acids. However, irrespective of the mechanism, it is evident that section of the entire vagal supply does markedly lower the concentration of the hydrochloric acid in the course of the normal digestive cycle. Progressive atony and dilatation occur following section of the vagus nerves if the animal is observed over a sufficient length of time, as has been shown by Lauwers, Dudek, and Beaver.

When the branches of the vagus nerves to the pyloric part of the stomach are sectioned, as was done on two of the animals in this series, the branches of the nerve to the fundus remain intact. Theoretically, there should be no retardation in the activity of the fundus. In each of these animals the emptying time was markedly reduced. Atony or dilatation did not occur. Authors who have reported dilatation and atony have cut the vagus nerves at a high point and in such a manner as to section the entire vagal supply to the stomach. It would seem probable that many of the variations in the results reported by different investigators with regard to both secretion and motility may be explained by the fact that they have cut the vagus nerves at different levels and have produced various types of denervation. Some observers have produced absolute discontinuity of the vagus nerves, and have left the sympathetic nerves intact, whereas others have performed denervation that included a relatively high proportion of sympathetic fibers. Another important factor is that certain

investigators have observed their animals only during the course of an acute experiment, others, over a period of two to four weeks, and still others, as long as one to three years.

SUMMARY

Experimental work on the effect on gastric acidity of section of the vagus nerves has been largely inconclusive for three reasons: 1, the preoperative normal state has not been adequately determined; 2, the observations often were made on animals that had been operated on for the production of fistulas and pouches, and 3, the period of postoperative observation has been too brief. The present study, which was carried out on dogs, is concerned only with the effect of section of the vagus nerves on the degree of acidity of the gastric content during that portion of the normal digestive cycle which begins with the ingestion of a meal and which ends when the stomach is empty. It was found necessary to train each animal for one to six weeks in order to have it thoroughly accustomed to the procedure. In the beginning, any psychic upset, even intubation, was sufficient to cause a marked decrease in acidity. Only at the end of the period of training did the acidity begin to show a uniform curve. Section of the vagus nerves then was performed. In some experiments the large anterior and posterior vagal trunks were cut just above the diaphragm; in others, the vagal branches were cut in the abdomen, care being taken to cut the pyloroduodenal nerve which descends from the vagal branch to the liver. In those dogs in which the vagus nerves were sectioned in the thorax, there was marked reduction in both free and total acidity, and the highest postoperative curve was lower than the lowest preoperative curve. There was one exception: in one animal the concentration after operation was the same as before operation, and at necropsy it was found that the posterior vagal trunk had been missed. In those animals in which the branches of the vagus nerves were cut in the abdomen the amount of free hydrochloric acid occasionally continued to rise to the same height as before operation. However, the secretory curve was shorter, so that the stomach was empty of all food and gastric juice, one to two hours sooner than before operation.

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STUDIES IN GLANDULAR METABOLISM[1]

I. The Source of the Lactic Acid Produced on Incubation of the Testicle and the Submaxillary Gland

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Several workers have shown that glands are capable of producing lactic acid on incubation but attempts made to discover its source have met with contradictory results. Embden and co-workers (1914) believed hexosephosphate to be the precursor of lactic acid since they found that the expressed juice of the testicle was able to break down hexose diphosphate into lactic acid and phosphoric acid, while Riesser (1926) reported that he had observed that NaF stimulated a synthesis of hexosephosphate in the liver. On the other hand, Schmitz and Chrometzka (1925) working with the submaxillary gland, parotid, and pancreas, and Krause (1927) studying the submaxillary gland, pancreas and liver, were unable to find any evidence for hexosephosphate as the source of lactic acid in glands.

Such varying results leave the question of the origin of lactic acid in glands as yet unsolved and fail to consider glycogen which has been demonstrated to give rise to lactic acid in muscle. Perhaps glands resemble muscle to the extent of having an anaerobic cleavage of glycogen with the formation of lactic acid, but differ from muscle in that hexosephosphate is not involved in the reaction. Barr, Ronzoni, and Glaser (1928) observed the occurrence of glycolysis without an intermediate hexosephosphate stage in malignant tumors. The following is a study of glycogen as a possible source of the lactic acid produced on incubation of the testicle and submaxillary gland.

Method. The procedure was to study both testicles or both submaxillary glands of an animal for their glycogen, intermediary carbohydrate and lactic acid contents, one gland being frozen and ground immediately on removal, the other, after mincing, being subjected to a period of incubation. The animals used were, in most instances, dogs though two experiments were performed on cats. In a few cases, diabetic dogs were used as they were available in connection with other work. The animals were

[1] Preliminary report. H. E. Himwich and S. A. Jacobson, Proc. Soc. Exper. Biol. and Med., 1927, xxv, 53.

either under amytal anesthesia or decerebrate. The gland that was first removed was weighed to 0.1 gram and then cut into small pieces in a 2 per cent Na_2HPO_4 solution. The minced gland was then placed in the incubator under relatively anaerobic conditions at 37°C. for two to three hours, after which a volume of 95 per cent alcohol, equal to twice that of the solution and gland together, was added. The second gland was removed, weighed rapidly to 0.1 gram and minced under cold alcohol in a mortar surrounded by cracked ice. Both glandular preparations were allowed to stand over night in alcohol and then examined for glycogen, intermediary carbohydrates and lactic acid.

The first step in the chemical analysis was the separation of the solid portion containing glycogen from the alcoholic solution containing lactic acid and soluble intermediary carbohydrates.

TABLE 1

Carbohydrate balance of pairs of glands—both frozen

NUMBER OF EXPERIMENT	WEIGHT OF GLANDS		GLYCOGEN		LACTIC ACID		INTERMEDIARY CARBOHYDRATES		TOTAL CARBOHYDRATES	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
	grams	*grams*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
1. Testicle	34.0	24.8	107	134 (HCl) (H-J)	64	72	12	15 (HCl) (H-J)	183	221
2. Submaxillary	9.1	8.9	83	67 (HCl) (S-H)	62	43	29	27 (HCl) (S-H)	174	137

Glycogen. The method used is essentially that of Pflüger (1909). After filtration, the residue is hydrolyzed with 60 per cent KOH and the glycogen precipitated by adding 95 per cent alcohol. On standing over night, the fluid portion is removed by filtration and the precipitated glycogen is dissolved in hot water. The glycogen is then hydrolyzed in 2.5 per cent acid and determined as glucose.

Intermediary carbohydrates. Large portions of the original alcoholic extract of the gland are taken for the determination of intermediary carbohydrates. Saturated ammonium sulphate is used to precipitate lipoids and such proteins as are not previously coagulated by alcohol or by heat, and the filtrate is hydrolyzed in 2.5 per cent HCl. Since glands have been found to contain reducing substances interfering with the determination of glucose (Folin and Berglund, 1922; Cori and Cori, 1924–25; Holmes and Holmes, 1925 and 1926), the extract containing intermediary carbohydrates is shaken with Lloyd's alkaloidal reagent after the acid hydrolysis as

recommended by Folin and Berglund (1922), and by Cori and Cori (1924–25). The Shaffer-Hartmann (1921) or the Hagedorn-Jensen (1923) method is used for the determination of glucose.

Lactic acid. Lactic acid is studied in small portions of the alcoholic extract after purification by the procedure as given by Meyerhof (1921). The final determination is made by the method of Friedemann, Cotonio and Shaffer (1927).

TABLE 2

Effect of incubation on carbohydrate balance

NUMBER OF EXPERIMENT	WEIGHT OF GLAND		GLYCOGEN		LACTIC ACID		INTERMEDIARY CARBOHYDRATES		TOTAL CARBOHYDRATES	
	Left	Right	Frozen	Incubated	Frozen	Incubated	Frozen	Incubated	Frozen	Incubated
	grams	*grams*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
1. Testicle	14.1 (inc.)	16.5 (froz.)	238	39 (HCl) (H-J)	18	199	13	26 (HCl) (H-J)	269	264
2. Submaxillary	9.4 (inc.)	9.3 (froz.)	198	34 (HCl) (H-J)	37	212	15	26 (HCl) (H-J)	250	272
3. Testicle	12.6 (inc.)	12.5 (froz.)	220	40 (HCl) (H-J)	36	344	16	25 (HCl) (H-J)	272	409
4. Submaxillary	4.5 (froz.)	4.2 (inc.)	133	29 (HCl) (S-H)	131	327	63	19 (HCl) (S-H)	327	375
5. Testicle	25.0 (inc.)	23.7 (froz.)	30	18 (H_2SO_4) (S-H)	29	279	19	34 (H_2SO_4) (S-H)	87	331
6. Testicle	11.5 (inc.)	11.3 (froz.)	225	37 (H_2SO_4) (S-H)	51	96	22	70 (H_2SO_4) (S-H)	298	203

RESULTS. In all the tables the method for the determination of reducing substance is indicated by the initials of the authors; H-J for Hagedorn and Jensen, and S-H for Shaffer and Hartmann. The acid used for hydrolysis is also indicated in the tables.

Table 1 shows the results of two control experiments in which both glands were frozen immediately upon removal. Three such observations were made on testicles, and four on submaxillary glands. It may be seen from the two typical experiments presented in table 1 that the total carbohydrate contents of each of a pair of glands did not differ significantly beyond the error of the method ($\pm$13 mgm. per 100 grams of tissue). In

one instance, a pair of submaxillary glands showed a larger difference in the totals (75 mgm. per cent), while in another case the total percentage values were the same in both submaxillary glands. In all the other experiments the variations were similar to those of the two examples given.

Fourteen experiments were done on testicles of dogs and two on those of cats while four observations were made on submaxillary glands of dogs. In all cases one gland was incubated to promote glycolysis, and the other frozen to prevent the formation of increased amounts of lactic acid. Six of these experiments are reported in table 2. As indicated, 2.5 per cent H_2SO_4 was used for the acid hydrolysis in some cases, while HCl was used in others. In five experiments (e.g., table 2, 1 and 2) a quantitative relationship was found to obtain between the decrease of glycogen and the increase of lactic acid, since the sums of the glycogen, intermediary carbohydrates and lactic acid were the same, within the error of the method in the frozen and the incubated gland. In nine other cases, of which experiments 3 and 4, table 2, are examples, a definite decrease in glycogen was found after incubation, but this decrease was less than the additional amount of lactic acid formed. In another group of four experiments (e.g., table 2, 5) there was a large increase of lactic acid in the incubated gland, although there was no significant decrease in glycogen. The amounts of glycogen in this last group were very small. In the 6th experiment of table 2, and in another similar to it, the loss of glycogen was greater than the additional lactic acid formed. In all but four instances a decrease of glycogen was observed after incubation, while an increase of lactic acid was demonstrated in every case.

In five observations (table 2, 6) an increase of more than 25 mgm. per cent of intermediary carbohydrates was found in the glycolyzed gland, though, in one instance (table 2, 4) the latter contained less than a third the amount in the frozen one. The remaining fifteen cases demonstrated no difference in the intermediary carbohydrates as a result of glycolysis.

Discussion. The results obtained indicate a decrease of glycogen and an increase of lactic acid in the testicle and submaxillary gland on incubation. In 13 observations, especially the earlier ones, performed while we were adapting the methods for the determination of glycogen, intermediary carbohydrates and lactic acid to the study of glands, the decrease in glycogen was insufficient to account for the increase of lactic acid. In the earlier experiments, the recovery of glycogen was probably incomplete since H_2SO_4 was used for hydrolysis to avoid the possibility of interference of the chloride ion with the copper reduction of the Shaffer-Hartmann (1921) method for estimating glucose. As it appeared that the glycogen was not completely hydrolyzed by 2.5 per cent H_2SO_4, in the later experiments the use of 2.5 per cent HCl was adopted, which in most instances produced a more complete recovery of glycogen. The ferricyanide method

of Hagedorn and Jensen (1923) for the determination of glucose was substituted for that of Shaffer and Hartmann (1921).

It seemed possible that another reason for incomplete recovery might be that portions of small amounts of glycogen (0.20–39.25 mgm.) were lost in the large volumes of fluid used in the determination, as suggested by Evans (1925). In the later experiments, a volume of 60 per cent KOH equal to one-half the weight of the gland was used instead of a volume equal to the entire weight. After the hydrolysis with KOH only sufficient water was used for washing (about 5 cc.) and then a volume of 95 per cent alcohol was added equal to one-half the total of the gland plus the KOH plus the water instead of twice the amount as suggested by Pflüger (1909).

The method used for the estimation of intermediary carbohydrates was also modified. It is possible that the use of both ammonium sulphate and Lloyd's alkaloidal reagent was unnecessary and furthermore caused a diminished recovery of glucose. Guerrant and Salmon (1928) have suggested that shaking with Lloyd's alkaloidal reagent removes glucose, though they found in accordance with our experience that when used in the presence of acid there is little adsorption of glucose. However, in the last experiment when neither ammonium sulphate nor Lloyd's alkaloidal reagent was used a value of 55 mgm. per cent was found for the intermediary carbohydrates before incubation and of 99 mgm. per cent afterwards. The latter result was higher than any other intermediary carbohydrate value obtained and the first was higher than the average. Four other experiments disclosed an increase of intermediary carbohydrates following incubation. It is possible that similar changes occurred in more experiments, but were masked by the analytical procedures. Boyland (1928) observed the same phenomenon on incubation of striated muscle of invertebrate animals.

In evaluating the significance of the changes in the various carbohydrates studied it must be remembered that hexosephosphate may be included in the determination of the intermediary carbohydrates (Embden and Jost, 1928). Unless these substances represent stages of cleavage between glycogen and lactic acid, it does not seem probable that they gave rise to the additional lactic acid produced on the incubation of glands, since in 19 of 20 experiments the intermediary carbohydrates did not decrease as a result of incubation. Moreover despite the earlier difficulties in the methods a quantitative relationship between the disappearance of glycogen and the production of lactic acid was demonstrated in five instances. In two other experiments, the decrease in glycogen was even greater than the increase in lactic acid. It seems probable then that the source of lactic acid was glycogen, even though we were unable to demonstrate this quantitatively in all cases.

SUMMARY AND CONCLUSIONS

After having observed that the percentages of total carbohydrates were approximately the same in each of three pairs of testicles and four pairs of submaxillary glands of dogs, a study was made of the effect of incubation on the glycogen, intermediary carbohydrate, and lactic acid contents of these glands. Sixteen pairs of excised testicles and four pairs of excised submaxillary glands were compared, one gland being subjected to glycolysis and the other frozen immediately upon removal.

In most instances there was no change in intermediary carbohydrates following glycolysis. Five experiments, however, offer evidence that incubation may increase the intermediary carbohydrate content to a slight though definite extent.

In five cases a quantitative relationship was found between the decrease of glycogen and the production of lactic acid; in nine experiments the decrease of glycogen, though marked, was less than the increase of lactic acid; in four other observations the glycogen, which was low in both glands, was unchanged on incubation; and in two cases the loss of glycogen was even greater than the increase of lactic acid.

The fact that most of the observations showed a decrease of glycogen indicates that this substance is the source of part if not all of the lactic acid formed on the incubation of the testicle and the submaxillary gland.

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THE RÔLE OF THE GLOMERULI AS THE PREFERENTIAL ROUTE FOR EXCRETION OF PHENOLSULPHONEPHTHALEIN IN THE FROG'S KIDNEY[1]

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Ever since Nussbaum (1878) took advantage of the double blood supply of the frog's kidney to study its secretion, this type of experiment has been performed repeatedly, and the results have been taken by both schools of kidney physiology, those adhering to the glomerular filtration theory, and those adhering to the tubular secretion theory, to substantiate their position.

The most commonly accepted view of the circulation in the frog's kidney is perhaps that described by Cushny in his Monograph, page 93 of the 1926 edition:

> In this animal, as in the mammals, the glomerulus is supplied by branches of the renal artery, and the efferent vessel breaks up into a second capillary system distributed over the tubules, from which the blood then passes into the renal veins. But in addition to this supply, the frog's kidney also receives blood from the renal-portal vein, which collects part of the blood from the lower extremities, trunk, and genital organs and carries it to the kidney where it is distributed through the meshwork on the tubules; the tubules are thus supplied with blood from two sources, the efferent vessels from the glomeruli and the renal-portal vein; these both empty into a common capillary meshwork and there is thus a complete mixture of the blood from the two sources. According to the usual view, these capillaries can be injected from either the renal artery or the renal-portal vein, and the tubules continue to receive a more or less adequate supply of blood if either the renal artery or the renal-portal vein is ligatured.

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The experiments herein reported form part of the thesis, "Contributions to the Physiology of the Kidney," submitted in May, 1929, to the Graduate School of the University of Minnesota by Raymond N. Bieter, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Nussbaum was the first investigator to take advantage of this double blood supply, and in his work attempted to prove tubular secretion. He injected sodium sulphindigotate into normal frogs and then killed the animals, removed the kidney and examined sections of the organ microscopically. He then compared these findings with those produced in a similar manner in kidneys, the renal arteries of which had been ligated. The normal kidneys showed the presence of the dye in the glomerular capillaries, tubular capillaries, and tubular lumen. In the kidneys of the frogs with ligated renal arteries, however, he found no dye in the glomerular capillaries, but did find it in the tubules and in the surrounding capillaries. He concluded that the tubular cells were actively secreting the dye into the lumen. He found, further, that carmine egg albumen and peptone were not present in the tubular lumina in these experiments; but on the other hand, if urea was injected intravenously into the frogs, where no urine was secreted before this procedure, about one-fourth of the normal urine volume appeared in which were found urea, chlorides and sulfates. Glucose injected in the same manner did not produce this flow of urine, but if it was injected along with urea, then it was to be found in the urine. Nussbaum concluded fom his experiments that the urine produced was secreted by the tubules.

Adami (1885) repeated the experiments of Nussbaum. Using carmine, after ligature of the renal arteries, he found it present in the glomerular capillaries, especially in the center of the kidney, but rarely at either end. The urine secretion from kidneys with ligated renal arteries was at first zero, but later increased, which Adami thought was due to a collateral circulation. In similarly prepared frogs, Adami also found hemoglobin in the capsule of Bowman, and it was also to be seen there when the blood pressure approached the venous level. From his experiments, Adami concluded that the glomerulus actively secretes the urinary constituents and that urea serves to excite the glomerular epithelium to increased secretion of water.

Beddard (1902) and Bainbridge and Beddard in 1906 again repeated this experiment. They reported the following results: 1, that ligature of the renal arteries permanently stops glomerular blood flow, because injection of the kidneys at the end of experiments showed no glomerular circulation; 2, that the tubular epithelium degenerates after this ligature; 3, that urea injections in the frogs produced no urine output, due, they think, to degeneration of tubular epithelium and absence of glomerular activity, and 4, that if frogs with ligated renal arteries were placed in an atmosphere of oxygen, tubular degeneration did not occur, and then following the injection of urea, urea and dextrose phloridzin or disodiumphosphate, urine was formed containing urea, chlorides, sulfates and acid. They concluded that in the frog, both the glomeruli and tubular epithelium

definitely secrete urine, and that in all probability, both can secrete much the same urinary constituents in solution. They further were of the opinion that when the quantity of urine was small, it was largely tubular in origin, but when the quantity was large, it was largely glomerular in origin.

A new technique was added to the study of the frog's kidney in 1906 when Miss Cullis first reported upon perfusion studies in the frog's kidney. Approximately the same results were obtained here as in the experiments of Nussbaum and Bainbridge and his co-workers, namely, that the glomeruli were not activated by flow of the perfusion fluid run into the kidney through the renal portal vein, and that the secretion of water, urea and sulfate, but not sugar, resulting from this perfusion, was a result of tubular secretion. No attempt was made, further, in these experiments, to determine the possibility of glomerular activity by injection studies. Again it should be borne in mind as Sollmann (1907) has shown for mammalian kidneys, that in perfusion experiments, death of the tubular cells results very quickly after the perfusion is begun. Schulten (1925) in a measure has confirmed this in that the tubular epithelium of frog's kidneys does not stain vitally in perfusion experiments. Lastly, these perfusion experiments of Miss Cullis, through the renal portal, were generally carried out at pressures of 25 to 40 cm. H_2O—a figure probably too high for this vessel.

This same type of perfusion has also been carried out by Rowntree and Geraghty (1910–12) and by Atkinson, Clark and Menzies (1921), with approximately the same findings. Rowntree and Geraghty also found phenolsulphonephthalein in the urine produced by renal portal perfusion. The same criticisms hold for the work of these investigators.

On the other hand, Bainbridge, Menzies and Collins (1914) concluded, from perfusion studies, that in the frog's kidney no urine was secreted if the glomeruli were not reached by the perfusion fluid, and that with relatively high pressures (28 to 30 cm. H_2O) renal portal perfusion reached the glomeruli. At pressures of 10 to 12 cm. of water they found that the great majority of glomeruli were not reached by renal portal perfusion.

Woodland (1922), working upon the Asiatic frog, described the blood flow through its kidney and claims that there is very little anastomosis between the renal arterial capillaries and the renal portal vein capillaries, and that the circulation through the arterial system only is responsible for the function of the organ.

In a previous communication (1924) in which the excretion of dyes was studied in frog's kidneys with normal circulation through both channels, and in kidneys in which part or all of the renal arteries were severed by cautery, we found that no dye collected in the lumina of the tubules unless there was an active glomerular circulation visible in the area of tubules showing dye within the lumen. For the most part, in the experiments

reported at this time, obliteration of the renal arteries resulted in a more or less complete cessation of glomerular blood flow, but in a number of areas in the region supplied by the ligated renal arteries, glomerular flow began again after one or more hours. The conclusion was drawn that this recurrence of glomerular flow in these areas was due to collateral circulation established, possibly, through the renal portal system.

Following the above report, the question of anastomosis between the renal portal and renal arterial systems in the frog's kidney, together with its relation to excretion of dyes, was studied during the ensuing two years.

To show the mixing of the pulsating arterial blood flow and the continuous renal portal flow, the following technique was found to be quite satisfactory. The renal portal vein coming from the lower extremity was ligated. This removed the greater source of blood flow from the kidney. The dorso-lumbar vein which joins the renal portal vessel at right angles on the lateral border of the kidney still shows a normal flow. If now the renal portal vessel along the lateral border of the kidney is brought into focus with the low power of a microscope, using Richard's method (1922) for illuminating the kidney (modified by the authors), the continuous flow of blood coming from the dorso-lumbar vein can be seen. In some cases a pulsating stream within the large renal portal vessel near its point of ligature can also be observed. The source of this pulsating stream has, as yet, not been determined. Following the renal portal branches out into the kidney, it could be seen that some of these branches, especially those nearest the point of ligature of the renal portal, showed pulsating streams, whereas those nearest the point of entry of the dorso-lumbar vein showed a continuous flow. Between these two types of flow, all variations were to be observed. Figure 1 shows a branch of the renal portal showing a mixture of these two flows. The pulsations observed were synchronous with the heart beat in all cases. The terminal branches of the renal portal all dipped into the kidney substance, so none could be followed to the direct site of anastomosis. Because of the fact that in most cases the pulsating flow was first to be observed in the very small branches, it was thought that the pulsations were transmitted by the heart through the larger arteries to the glomerular vessels and thence to the smaller veins, and lastly, to the larger branches of the renal portal system. These observations were carried out in the kidneys of five frogs of the species Rana pipiens.

In two other frogs, every visible renal artery was severed by cautery. When these kidneys were observed under the microscope, a few small arterioles showing pulsating streams running into the kidney substance, the pulsations of which were synchronous with the heart beats, were to be observed in the lower one-third of the kidney, ventral surface, and also in the upper pole. No glomeruli showing active flow were observed near

these arterioles. The blood flow in the rest of the kidney was slow and continuous.

To study the secretion of the kidney when a substance is supplied to the kidney through the renal portal only, and to study its relation to glomerular activity, the following technique was devised. It was noticed in perfusion experiments, simultaneously through the renal portal vein and renal arteries, that after the addition of a dye to the renal portal perfusate, when the dye was present in the urine in the ureter in a sufficient concentration to be visible to the naked eye, that the portion of the ureter along the upper half of the kidney did not appear to show as deep a coloration as the lower portion of the ureter. The question was then raised as to whether or not this apparent difference in the amount of dye elimination in the two halves of the kidney in this type of an experiment, might not

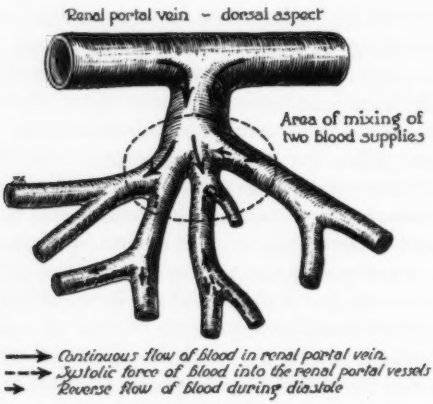
Renal portal vein - dorsal aspect
Area of mixing of two blood supplies
Continuous flow of blood in renal portal vein.
Systolic force of blood into the renal portal vessels
Reverse flow of blood during diastole

Fig. 1

be associated with more and less of the glomeruli receiving blood by an anastomosis from the renal portal vein in the lower and upper halves of the kidney, respectively.

Therefore, studies were made of the secretion of phenolsulphonephthalein by the upper and lower halves of the kidneys of Rana catesbiana. In the series of frogs described in tables 1 and 2, one frog only was used for each experiment. One cannula was inserted into the coeliac artery by means of which the renal arteries could receive oxygenated Ringer's fluid at a pressure of 25 to 35 cm. H_2O. (It was found in several frogs used at this time, that this corresponded to the systolic coeliac blood pressure.) By means of two other connected cannulae, one inserted into one branch of the aorta, and one inserted into the common iliac artery just below the bifurcation of the aorta, aortic blood could be shunted over the kidneys

and into the leg, from which it was collected and run into the kidney by means of the renal portal vein on the same side. During the surgical procedure necessary for the above manipulations, the other branch of the aorta carried blood from the heart to the kidneys, thereby preventing any serious lack of oxygen, and its resulting consequences, to the kidney. Two other cannulae were now inserted into the upper and lower ends of the ureter. The insertion of the upper cannula in some frogs was found to be impossible, but in others the ureter extended a few millimeters above the

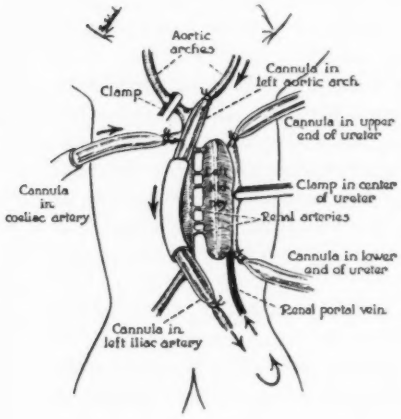

Fig. 2

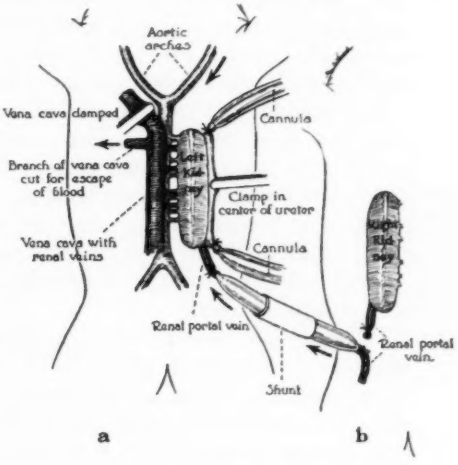

Fig. 3

Fig. 2. Diagram showing method of "shunting" aortic blood from one aortic arch around kidney. This allows the blood to run into kidney through the renal portal vein. Oxygenated Ringer's fluid is run into kidney through renal arteries by means of coeliac artery. Renal veins collecting these two fluids and transporting them to vena cava are not shown.

Fig. 3. Frog *a*. With kidney used for experiment using own renal arterial blood. Frog *b*. Supplying renal portal blood to kidney of frog *a*.

kidney, and in these animals the procedure was not particularly difficult. When the ureter in these frogs was clamped in the center, the urine from the upper and lower halves of the kidney could be collected separately. Thus it can be seen that by this method a very satisfactory arrangement can be obtained by which substances can be injected into the kidney from either source of blood supply, and the urines from the two halves can then be collected separately. At the same time, while the preparations are being made, the kidney is not suffering, at least, to any great extent, from lack of oxygen. Figure 2 shows a diagram of this technique.

When the preparation of the animal is completed, the shunt between one of the aortic arches and the iliac is opened and the other aortic arch is clamped. Then oxygenated Ringer's is allowed to run into the coeliac and flow into the renal arteries. Thus the renal arteries cannot receive any of the animal's blood being pumped through the body by the heart. By means of the renal portal supplied by the shunted blood, however, the kidney receives blood. Under these conditions the urine volumes from the two halves of the kidney have been found to be about equal. During these experiments the kidney at no time has appeared anemic or cyanotic, providing only active frogs with satisfactory cardiac contractions and good respiratory activity were used. These conditions held in this series of frogs, as well as in the other series of frogs used.

The first series of frogs so studied are shown in table 1. Immediately after the shunt was opened and oxygenated Ringer's run into the renal arteries, an injection of 0.1 cc. of phenolsulphonephthalein was made slowly into the shunt carrying blood around the kidneys to supply the renal portal vein through the leg. This dye solution was made up according to Clark (1922) and was of a 0.4 per cent concentration. In the last five frogs of this series, the dye solution was diluted $\frac{1}{2}$ to 1 cc. with saline, and then slowly injected.

Following the administration of phenolsulphonephthalein, the time for the appearance of the dye in the urine was noted. It appeared in the urine in the cannulae in the lower part of the ureter (lower half of kidney) within fifteen minutes in every case, and in the first nine frogs it was present in this cannula at the end of five minutes. Its appearance in the upper cannula (from upper half of the kidney) was slightly slower. It required, as a rule, ten to fifteen minutes in most cases.

The amounts of phenolsulphonephthalein secreted by the two halves of the kidney are shown in each case. One + indicates a faint trace and in each frog successive samples of urine were compared to this first trace and the amount of dye computed from this. For example, ++ means, upon dilution, twice as much dye as is indicated by +. The first urines collected were either slightly alkaline or neutral to the phenolsulphonephthalein contained in them. As the experiment progressed, the urines tended to become more acid. The dye amounts secreted by the two halves of the kidney were compared with each other for each frog and bear no definite relation to the amounts of dye secreted by the kidneys of the other frogs. In other words, this table shows merely that in each case the lower half of the kidney put out from two to six and eight times as much dye as the upper half of the kidney. In frogs 2 and 3 only did the urines of the upper half of the kidney show more than a trace of dye, and in these two cases it was found by the rough colorimetric determinations described above, that the amounts in these two cases were about

TABLE 1

The excretion of phenolsulphonephthalein when injected into the renal portal blood stream, and the percentage of glomerular injection produced by similar injection of American india ink

FROG NUMBER	WEIGHT	TIME PHENOLSULPHONEPHTHALEIN (0.1 cc.) INJECTED	URINE SPECIMENS COLLECTED					GLOMERULI INJECTED WITH INDIA INK	
			Time	Upper half of kidney		Lower half of kidney		Upper	Lower
				Amount of dye	Urine volume	Amount of dye	Urine volume		
	grams				*cc.*		*cc.*	*per cent*	*per cent*
1	550	10:00	10:15	+		++			
		10:15	11:00	+		+++			
2	500	11:30	11:35	0	0.1	+	0.1		
			11:50	++	0.2	++++++	0.3		
			12:00	+	0.1	++++	0.1		
3	640	11:10	11:20	+	0.03	++++	0.05	8	34.0
			11:50	++	0.1	+++++++++	0.1		
			12:05	+	0.15	++++	0.2		
4	600	10:40	10:50	+	0.1	+++	0.15		
			11:10	+	0.3	+++	0.4	28.5	46.0
5	600	11:10	11:20	0		+			
			11:30	+		+++			
			11:50	+		++++++			
6	300	3:45	3:55	+	0.18	+++	0.2		
			4:05	+	0.1	+++	0.1	9	17.5
7	280	9:00	9:05	0		+			
			9:15	+	0.1	+++	0.1	19.4	37.6
8	270	10:00	10:15	+	0.1	++	0.1		
			10:30	+	0.1	+++	0.1	18.0	45.0
9	200	12:00	12:12	0	0.1	+	0.1		
			12:30	0	0.15	+	0.15	8.7	42.0
10	350	10:50	10:55	0	0.1	0	0.1		
			11:05	0	0.05	+	0.05	4.3	24.7
			11:20	+	0.2	+++	0.3		

+ = trace of phenolsulphonephthalein.

twice the amount of a so-called trace for these two frogs. As can also be seen from this table, the urine volumes from both halves were approximately equal.

Following the study of dye secretion by these kidneys, Higgins' American india ink was injected into the aortic-iliac shunt. The particles of ink were then carried into the kidney by means of the renal portal and they were deposited in the glomeruli through which the renal portal blood flowed. The kidney was allowed to receive renal portal blood containing india ink for periods of from one to fifteen minutes. Following this, the kidneys were rapidly removed and placed in formalin. Microscopic sections stained slightly with eosin were then made of both the upper and lower kidney halves at different levels, and the number of glomeruli showing india ink deposits were counted and compared with the total number of glomeruli.

In the cases of the first two frogs, the india ink was allowed to flow into the kidney for only two or three minutes. Grossly, this exposure to ink resulted in the kidney turning almost completely black, but on microscopic examination, no black deposits were to be found in the glomeruli. In the light of the following results, it was thought that in these two cases the ink was not run in for a sufficiently long time to reach the glomeruli. This is pointed out as a possible source of error in other smilar types of glomerular injection studies. In the cases of the other kidneys (frogs 3 to 10) the blood containing ink was allowed to flow into the kidneys for from ten to fifteen minutes. In these kidneys (with the exception of frog 5, the slides of whose kidney were lost) the india ink showed beautiful glomerular capillary injection. In some cases, what appeared to be all of the capillaries showed a fine black deposit; in other cases, only one or two or three capillaries showed this black deposit, and in other fewer instances, the glomeruli showed rather coarse droplets of the ink. This variation in the number of capillaries showing the deposit is thought to be due to the fact that in some glomeruli only part of the capillaries show active flow as was first pointed out by Richards (1922) and is here, indirectly, again confirmed. It must also be pointed out that in studying these sections of the kidney, in over seventy-five per cent of the kidneys the tubular capillaries of both the upper and lower halves showed a marked india ink injection, especially on the dorsal surfaces.

In a second series of frogs, similar observations were made and recorded, but with the following changes in technique. The dye solution, instead of being injected over one relatively short interval of time, was injected continuously over a period of from ten to fifteen minutes. This solution of phenolsulphonephthalein contained 0.1 cc. of the stock solution (0.4 per cent) per 50 cc. of Ringer's fluid. Injecting continuously at a rate of 1 cc. per minute, the kidney was supplied with the dye more evenly, and again starting with a definite concentration, the urine samples could be compared with it to determine the concentrating power of the kidney during the experiment.

TABLE 2

The excretion and concentration of phenolsulphonephthalein injected into the renal portal blood stream, and glomerular injection produced by similar injection of American india ink

FROG NUMBER	WEIGHT	DYE INJECTED		URINE SPECIMENS COLLECTED							GLOMERULI INJECTED WITH INDIA INK	
					Upper half of kidney			Lower half of kidney				
		Amount	Time began	Time	Dye output	Concentration	Urine volume	Dye output	Concentration	Urine volume	Upper	Lower
	grams	*cc.*					*cc.*			*cc.*	*per cent*	*per cent*
1	530	15	11:00	11:05	0			+				
				11:10	0			+	½ ×			
				11:20	+			+	1 ×		16.8	24.5
				11:25	+			+	2 ×			
				11:30	+			+	3½ ×			
2	570	10	4:35	4:40	0		0.01	+		0.05		
				4:45	0		0.02	+	½ ×	0.02		
				4:50	0		0.03	+	¾ ×	0.07	32.0	97.5
				4:55	0		0.03	+	1 ×	0.1		
				5:05	+		0.1	+	5 ×	0.15		
3	470	5	2:45	2:50	0		0.05	+		0.05		
				2:53	0		0.03	+	½ ×	0.03		
				2:56	+		0.03	+	2 ×	0.04	14.4	33.0
				3:01	+		0.03	+	3 ×	0.04		
				3:06	+	½ ×	0.04	+	2 ×	0.05		
4	490	15	3:05	3:10	0		0.01	0		0.01		
				3:15	0		0.01	+	½ ×	0.01		
				3:20	0		0.04	+	2 ×	0.05	14.5	56.5
				3:22	+		0.03	+	3 ×	0.05		
				3:24	+	¾ ×	0.03	+	2 ×	0.04		
5	540	10	3:10	3:15	0		0.02	0		0.02		
				3:20	0		0.04	+	½ ×	0.07		
				3:26	0		0.06	+	1 ×	0.08	9.0	65.0
				3:29	0		0.02	+	2 ×	0.03		
				3:34	0		0.03	+	3 ×	0.03		
6	570	10	11:25	11:30	0		0.05	0		0.03		
				11:35	0		0.04	+		0.02		
				11:40	+		0.05	+	¾ ×	0.02	16.5	63.0
				11:45	+		0.08	+	1 ×	0.07		
				11:50	+		0.08	+	3 ×	0.05		

1 × = same concentration of dye as in injected solution.

In the first three frogs of this series, the dye solution was injected into the anterior abdominal vein, and in the last three, into the pelvic vein which drains directly into the main renal portal trunk. Because of the fact that two separate solutions were being injected into the frogs and there was this danger of "water-logging," a branch of the vena cava was cut soon after the beginning of the experiment to prevent two great a venous pressure from developing. Furthermore, the observations were carried out as quickly as possible in each frog, the average duration of the study of the secretion of phenolsulphonephthalein for the six frogs being twenty-five minutes. Two were completed in twenty minutes.

The results of this study are shown in table 2. The phenolsulphonephthalein solution injections varied from 5 to 15 cc. In the first frog, American india ink, 0.3 cc., was added to the dye solution at 11:20 and this was allowed to run in for ten minutes. In the remaining frogs in the series, the same amount of ink was added to 10 cc. of the dye solution at the start, so in these frogs the kidney was exposed to the india ink throughout the observations.

As can be seen from table 2, the dye again appeared in the urine, first from the lower half of the kidney within five to ten minutes, and then from the upper half on an average slightly over eighteen minutes, after the injection was begun. It is thought that this rapid elimination of the dye speaks for a kidney very close to normal.

Following its appearance in the urine from the lower half of the kidney, it can be seen, from the tables, that it rapidly arose in concentration and before long the urine showed phenolsulphonephthalein in a concentration greater than that in the original solution injected. In every case the lower half of the kidney was able to concentrate the dye to from three to five times the concentration of the dye injected. These comparisons were made, as before, in very small test-tubes constructed out of glass tubing. It can also be noted from the table that the urine volumes from the two halves of the kidney ran fairly uniformly.

On the other hand, the upper halves of the kidneys, not excreting as large an amount of the dye, did not show a concentration greater than that of the solution injected. In frogs 3 and 4, the concentrations amounted to one-half and three-quarters of the original. In all probability this represents a concentration, however, because the dye solution injected was mixed with at least an equal volume of blood and probably a volume up to twice the volume of the solution injected, which was 5 cc. (In the blood protein studies of Bieter and Scott (1929) it was relatively easy to draw 6 to 8 cc. of blood from a frog of this size, in a very few minutes.)

At the end of the experiments (represented by the last time for a collection of urine from the kidney), the kidneys were rapidly removed and placed in formalin. Sections of these halves of the kidneys, stained slightly

with eosin, were again prepared and studied for the percentage of injected glomeruli and the extent of the tubular capillary injection.

Finally, another series of kidneys were studied in which a separate blood flow for both the renal arteries and renal portal vein was maintained. It was thought worth while to do this to remove the possible criticism of perfusion studies. Two frogs were used for each experiment. The frog whose kidney was studied supplied arterial blood to the renal arteries of its kidney (frog *a* in fig. 3). By means of two cannulae, one in the proximal end of the renal portal vein of the same frog, emptying toward the kidney, and one in the distal end of the renal portal vein of the second frog taking the renal portal blood from the branches below the kidney, the renal portal blood of the second frog (frog *b* in fig. 3) was carried through the connected cannulae to the kidney of the first frog. Thus two separate blood supplies entered the kidney studies at about normal blood pressures. The solution containing phenolsulphonephthalein and india ink in the same concentrations used above was injected into the pelvic vein of the second frog, so that it would flow into the renal portal blood and be carried by the shunt over to the kidney to be studied in the first frog. This series of experiments, then, is identical with those preceding except that blood was run into the kidney instead of oxygenated Ringer's fluid. Figure 3 shows a diagram of this technique.

In this series of animals the vena cava in the frog whose kidney was excreting the dye, was also clamped, and a small branch was then incised toward the kidney from the clamp. This was primarily to prevent the renal venous blood, which also contained the renal portal blood plus dye from the second frog, from reaching the heart where it would then be pumped out into the arteries and then reach the renal arteries. In other words, the dye was allowed to reach the kidney, as in the other series of frogs, only through the renal portal system. The blood was therefore only run through the kidney once, after which it was allowed to escape. To prevent rapid bleeding of the frogs, oxygenated Ringer's solution was injected slowly into the anterior abdominal veins of both frogs. This provided sufficient fluid for an ample blood pressure for the duration of the experiments.

The results obtained are shown in table 3. Here, again, the dye appeared in the urines coming from the lower halves of the kidneys sooner than it did in the urines from the upper halves of the kidneys. In the first six frogs, moreover, the lower urines showed the dye to be concentrated from $1\frac{1}{2}$ to 6 times that in the solution injected. The upper urines in three of the first six frogs showed the dye in one as a definite trace, but in the other two, in concentrations $1\frac{1}{2}$ to 2 times that in the injected solution, but at a time when the lower urines were showing concentrations five and six times that of the solution injected, respectively. That these kidneys were

able to excrete the dye and concentrate it rapidly, speaks for kidneys functioning normally.

TABLE 3

The excretion and concentration of phenolsulphonephthalein injected by means of the renal portal vein and per cent glomerular injections by the same route in frogs' kidneys receiving two separate blood supplies

FROG NUMBER	WEIGHT	TIME DYE (0.1 cc.) INJECTED	URINE SPECIMENS COLLECTED							GLOMERULI INJECTED WITH INDIA INK	
				Upper half of kidney			Lower half of kidney				
			Time	Dye output	Concentration	Urine volume	Dye output	Concentration	Urine volume	Upper	Lower
	grams					*cc.*			*cc.*	*per cent*	*per cent*
1	350	11:00	11:15	0		0.1	+		0.1	8.4	29.7
			11:30	+		0.1	+	¾ ×	0.1		
2	540	11:00	11:15	0		0.1	+		0.15		
			11:30	0		0.05	+	2 ×	0.10		
3	600	3:36	3:38	0		0.03	0		0.02		
			3:42	0		0.04	+		0.04	19.2	78.4
			3:47	+		0.04	+	1½ ×	0.05		
			3:52	+		0.05	+	2 ×	0.06		
			3:56	+	1½ ×	0.02	+	5 ×	0.05		
4	360	10:13	10:16	0			+				
			10:20	0		0.04	+	1½ ×	0.05	14.0	44.5
			10:24	+	2 ×	0.04	+	3½ ×	0.04		
			10:30	+	2 ×	0.07	+	6 ×	0.08		
5	260	2:20	2:25	0		0.05	0		0.05		
			2:30	0		0.05	+	2 ×	0.05	7.8	61.1
6	270	2:40	2:45	0		0.04	0		0.04		
			2:55	0		0.08	+	1½ ×	0.09	6.0	40.0
7	560	1:40	1:45	0		0.05	0		0.05		
			1:55	0		0.05	0		0.05	0	1.7
8	620	3:10	3:15	0		0.05	0		0.04		
			3:25	0		0.1	0		0.1	0	1.0
9	530	12:40	12:45	0		0.07	0		0.05		
			12:50	0		0.05	0		0.05	0	0.5
			12:55	0		0.04	0		0.04		

The urines collected from the last three frogs failed to show the presence of the dye at any time during the time of the experiment, yet both were

excreting urine volumes comparable to those obtained above. In all cases, again, the urine volumes approximated each other fairly well from the upper and lower halves of the kidney.

At the time of the last recorded collection of urine for each kidney, the kidneys were again removed quickly and placed in formalin. Sections were prepared as before, and the injected glomeruli and tubular capillaries were studied.

The results obtained from the study of the kidney sections, upper and lower halves separately, have been very much alike in the three series, consequently they will now be considered together. In the first place, india ink run into the kidney for only two or three minutes has failed to show any glomerular injection in these kidneys (frogs 1 and 2, table 1). When, on the other hand, the india ink was allowed to run into the kidney for a period of at least ten minutes, the pictures produced were striking. The sections of the lower halves of the kidneys, except in frogs 7, 8 and 9, table 3, which will be considered separately, always showed a marked injection of the glomerular capillaries. The lowest percentage of injected glomeruli was obtained in frog 6, table 1, which was 17.5 per cent, and the highest percentage was that in frog 2, table 2, namely, 97.5 per cent. The total average (excluding frogs 7, 8 and 9, table 3) was 46.6 per cent for the number of glomeruli in the lower half of the kidney that can be injected by means of the renal portal circulation.

The upper halves of the kidneys, on the other hand, did not show as marked an injection. The lowest percentage obtained here was 4.3 in frog 10, table 1, and the highest was 32 per cent in frog 2, table 2, giving an average again for the three series of 14.4 per cent.

The injection with india ink of tubular capillaries has also been noted. In most of the cases these vessels have shown a marked injection, especially of the capillaries around the proximal convoluted tubules on the dorsal sides of the kidneys. In a few there was somewhat less tubular capillary injection in the upper half of the kidney as compared with the lower half, but this difference was not great.

These injection studies show that the intertubular capillaries in the frog's kidney can be injected very easily through the renal portal vein. The glomerular capillaries can also be injected through this vein with ease. In the lower halves of the kidneys, this injection is most intense. However, a certain amount of injection of the glomerular capillaries in the upper halves also occurs and is always to be found in those kidneys the lower halves of which show an appreciable injection.

In frogs 7, 8 and 9, table 3, it can be seen that there was practically no injection of the glomeruli in either the upper or lower portions of the kidneys. This was observed, despite the fact that the tubular capillaries showed marked injections. Apparently every frog does not show the same

degree of glomerular injection by way of the renal portal and this probably means that every frog does not show the mixing of the renal arterial blood and the renal portal venous blood in the glomerular capillaries, as completely as some others do. Just why this variation exists is at present unknown.

It is also important to notice in these three frogs that the urines—although their volumes were about equal to those obtained in the other frogs—showed no phenolsulphonephthalein. This is the most important observation recorded in these three frogs. It shows that their kidneys excreted no appreciable amounts of the dye because the blood containing the dye did not reach the glomerular capillaries.

Turning now to the kidneys of the remaining frogs in these three series, it can be seen that the lower kidney halves excreted the dye in every case. In every animal, moreover, the dye appeared in the urine quickly after the injection and in relatively large amounts. This elimination came from the kidney halves showing an average glomerular injection of 46.6 per cent. In the upper halves these same relationships are shown just as strikingly. The average glomerular injection in these halves was 14.4 per cent, and in the urines of eighteen out of twenty-two of these same kidney portions, phenolsulphonephthalein was found, but only after slightly longer intervals of time and in smaller amounts.

The same conclusions can therefore be drawn from these experiments, namely: that rapid elimination and concentration of phenolsulphonephthalein depend upon the dye reaching the glomerular capillaries. In other words, the dye must be carried by the blood to the glomeruli, where rapid elimination begins; then as this fluid runs down through the tubules, it appears that water is re-absorbed and the urine then appears in the ureter and ureteral cannula, showing dye in a concentration greater than that of the solution injected. If the tubules were able to secrete phenolsulphonephthalein in an amount that could be seen in the urine specimens with the naked eye, or in an amount comparable at all with that which in these experiments depends upon the dye solution reaching the glomerular capillaries, it should have appeared in the urines of frogs 7, 8 and 9, table 3, which, however, it did not do. In these three frogs, it should be remembered, urine was excreted, but in all probability this came from the renal arterial blood supplying the glomerular capillaries and not the renal portal blood, which in these experiments, always contained the dye.

Discussion. The findings herein reported on the ability to inject the glomeruli through the renal portal help to elucidate many apparent discrepancies in the work upon the excretion of various substances, especially dyes, in the frog's kidney. In the first place, the tubular capillaries throughout the kidney are easily injected by means of the renal portal, thus showing, as was first pointed out by Nussbaum (1878), that the two

systems of blood flowing into the kidney show a complete mixing in these vessels. (Injections of india ink made by means of the renal arteries in frogs have likewise shown a marked tubular capillary injection in this laboratory.)

In the second place, the glomeruli can easily be injected through the renal portal as was first shown by Adami (1885) and therefore the criticisms advanced by this worker against renal portal injection of substances with conclusions of tubular secretion, still hold. Furthermore, if glomerular injections are attempted by this route for a period of only two or three minutes, the conclusions drawn from them are not apt to be justified, because glomerular injection within this short space of time is apt to fail, as was demonstrated in frogs 1 and 2, table 1. These findings also confirm those of Bainbridge, Menzies and Collins (1914) who showed that when the renal portal system in frogs was perfused, urine was excreted only when the glomeruli were reached by the perfusate. The fact that in the series of frogs reported here, three were found that failed to show glomerular injection and excretion of phenolsulphonephthalein from the renal portal, indicates that in some frogs this anastomosis between the two systems is not as marked as in others. Thus, these findings may then help to explain the varying reports in the literature on the ability of the frog to put out urine when its kidneys receive only renal portal blood. On the other hand, the findings in these three frogs might point to an incompatibility between the bloods of the two frogs; yet the fact that all three excreted urine points against this; and lastly, it might be explained by too low a pressure in the renal portal to cause glomerular filtration, and again, the fact that all three excreted urine points against this.

The findings of Woodland (1921) and also of Smith (1927) who reported his results after the preliminary reports of this work were read, are not confirmed in this paper. Yet it should also be added that Smith apparently did not attempt to produce and look for glomerular injection via the renal portal vein.

Bensley and Steen (1928) have repeated the famous experiment of Nussbaum and have apparently confirmed his findings. They have however failed to prove that the glomeruli were completely inactivated throughout their experiments. They mentioned in one case (p. 93) that the glomeruli appeared congested and bright red. It might be added at this point that in the studies on kidney function in this laboratory, which have been running for six years or more, it has been repeatedly observed that when a glomerulus shows complete and final stasis, the color of the red cells changes very soon to one of deep purple cyanosis and does not remain bright red. This would therefore tend to indicate blood flow in some of their glomeruli. Again, "plasma skimming" has been beautifully demonstrated for frog's glomeruli by Richards and Schmidt (1924) and it has been repeatedly

confirmed here. This again might have played a rôle in the experiments of Bensley and Steen. Lastly, these authors state that it required from forty minutes to four hours in their experiments, for the dye injected (in doses which they do not give) to appear in the lumen of the distal convolutions. The question is now asked: Does this elimination, requiring almost an hour for a visible amount of the substance to appear in the tubular lumen, appear to be the result of a normal important excretory function in the frog's kidney as compared with a marked and visible macroscopic elimination of dye dependent upon glomerular activity as has been demonstrated herein to occur within five minutes of the injection into a vein?

This elimination of dyes as described by Bensley and Steen which, on the other hand, may be the same as that described by Edwards and Marshall (1924), appears in the light of the work of Richards and Barnwell (1927) to be due to what they call diffusion of the dyes through the kidney substance. This diffusion requires a relatively long time for its development and tends to be primarily into the lumen of the proximal convolutions where the fluid is then propelled, probably by the cilia, into the distal convolutions and there concentrated. This concentration is furthermore prevented with sodium cyanide. Thus it appears that the work of Bensley and Steen is more of a confirmation of this so-called diffusion of dye and fluid into the proximal convolution, with moving of this fluid into the distal convolution, where it is concentrated by reabsorption of water, than it is a proof of an important normal part of kidney function in the frog.

The experimental work on the injection of the glomeruli through the renal portal vein has been confirmed since the preliminary reports were published in 1925–26, by Richards and Walker (1927) and Hayman (1928). They all confirm the observation that the glomeruli can be injected by means of this vein. Smith (1927) also confirms the findings of the complete mixing of the renal portal and arterial blood in the tubular capillaries. Richards and Walker have further elaborated upon this question and find that many glomeruli can receive fluid from both the renal arteries and renal portal vein by perfusing differently colored solutions simultaneously through these two channels. This shows still further the complete mixing of the two bloods in the frog's kidney. Their figures for the percentage of glomeruli injected through the renal portal are very like those reported in this paper.

Richards and Walker also report that when perfusing the renal portal vein at a pressure of from 10–12 cm. H_2O, no urine is formed. This brings up the question of whether the normal renal portal pressure is high enough to cause glomerular filtration. Hayman (1928) reported that the average renal portal pressure from one hundred one estimations was 5.2 cm. H_2O, and that this figure is below the osmotic pressure of the proteins as

given by White (1924) and Landis (1928). Several determinations of renal portal pressure made in this laboratory in 1925 have indicated the pressure to be from 10 to 15 cm. H_2O, or equal to about 11 mm. Hg, for the high figure. Taking the plasma protein percentages of Bieter and Scott (1929) as 3.8, this would give an osmotic pressure also of 11 mm. Hg. Whether in the frogs herein studied, excretion of urine in the glomerulus was purely filtration, in which case dilution of the blood by the saline injected during the experiment would be necessary to lower the protein percentage and therefore its osmotic pressure, or whether the pressure in the renal portal vein was higher in these experiments than the above measurements would indicate, or whether it is necessary to add to glomerular filtration the conception of "glomerular secretion," still remains to be proven. This idea of glomerular secretion was first advanced in a theory of kidney function by the early American physiologist, Charles Edward Isaacs, in 1857, and was later described independently by Adami (1885) and was recently accepted by White (1928) to explain his findings. McGregor, in a recent study of the membrane surrounding the glomerular tuft within the capsule of Bowman, has found a basement membrane and upon it a cellular structure, which would offer no objections to such a theory.

In an attempt to harmonize all of the results of the recent studies by Richard's method, of kidney function, the work of Marshall and his coworkers upon the aglomerular kidneys of fish should not be overlooked. These aglomerular kidneys have practically no glomeruli or else very few that appear to be functionally important. Marshall and Grafflin (1928), Edwards (1928) and Edwards and Condorellin (1928), have reported upon studies of the structure and function of kidneys that are aglomerular as well as upon others that are only partly aglomerular.

Marshall and Grafflin (1928) studied the kidneys of the fish, Lophius piscatorius. From serial sections and maceration studies of these kidneys, these investigators have estimated that they contain about 150,000 tubules per kidney as a minimum, and about one pseudo-glomerulus per 2000 tubules. These pseudo-glomeruli of which they found about 80 per kidney, consisted of loops of capillaries receiving arterial blood and invested with a rather large, irregularly shaped Bowman's capsule. Functional connections between these capsules and tubules were very difficult to demonstrate. Associated with these structures were areas of sinusoidal tissue, described for the first time, by these authors, and also receiving arterial blood. Both the pseudo-glomeruli and sinusoidal tissues were found to be filled with coagulated fluid.

The tubules of these kidneys appeared to end blindly in practically every case. A diagram showing fifty-four different endings is included in their paper. The convolutions of the tubules appeared to show no

uniformity in their locations, and the epithelial lining of the tubules showed no areas of differentiation but was fairly uniform throughout. The greater part of the blood supply to these kidneys, or perhaps more correctly, to the tubules, is venous in character, undoubtedly analogous to the renal portal blood flow in the frog.

Marshall and Grafflin have also found that this fish is able to excrete from 500 to 700 cc. of urine in 20 to 22 hours. A table of serum and urine contents is given. One of the most unusual findings was that the urea, uric acid and creatinine N in the urine was very low, despite the fact, as they say, that the fish lives primarily upon protein. Studying the injections of various foreign substances, they discovered the remarkable fact that following the intravenous injection of glucose or ferrocyanide, neither of these two substances appeared in the urine. Lastly, they studied the excretion of phenolsulphonephthalein injected intravenously, intracardially and intraperitoneally. The dye appeared in the urine in the three experiments 30, 38 and 72 minutes after the injections. Compared with the concentrations in the blood plasma, the urine phthalein was concentrated up to 8 and 28 times in two cases. The doses of the dye administered were 12 and 72 mgm. for two of the animals. These were considerably larger, especially the latter, than those used in the frogs in this investigation (0.4 mgm. per frog).

Edwards and Condorelli (1928) have also studied the excretion of dyes by the kidneys of various fish. They described their findings of excretion of tetrachlorphenolsulphonephthalein in four fish,—two being aglomerular, one practically aglomerular and one showing 50 to 60 per cent glomeruli. Their percentages of excretion for the kidneys for a period of 15 to 18 hours in three of the fish are, on an average, 24 and 28 per cent for the two aglomerular, 72 per cent for the 50 per cent glomerular kidney, and for the practically aglomerular kidney, 20 per cent at the end of thirty minutes.

In discussing their findings, Marshall and Grafflin bring out the point that the elimination of phenolsulphonephthalein by these aglomerular kidneys may be an excretion across the tubular epithelium not involving secretion as we ordinarily consider that term. This appears to be of extreme importance. In the frog's kidney, Richards and Barnwell (1927) have shown, as was stated previously, that this dye will slowly get into the lumen of the proximal convolutions by what they term diffusion. These two processes may be entirely alike and are in all probability not secretory. It should also be remembered that the mammalian kidney will not "secrete" against a pressure in the ureter, like a salivary gland against a pressure in its duct (see Cushny, p. 140, for discussion).

Obviously, however, an aglomerular kidney must eliminate its urine through its uriniferous tubules unless nephrostomes are shown to play an important part. Regardless of the function of the latter, it must be borne

in mind that the tubules of these kidneys are of a more simple structure than is the case even in the frog. This more primitive structure would tend to indicate less specialization of function by different parts of the uriniferous unit.

The fact that these kidneys eliminate phenolsulphonephthalein very slowly, whereas the glomerular kidney of the frog can eliminate and concentrate the dye with much greater rapidity, speaks for the following conclusion: *that the preferential method of excretion by means of the kidney is the primarily glomerular route.* Just how important this tubular elimination is in kidneys with glomeruli, as for example in the frog, cannot be decided at present. It appears from the work of Richards and Barnwell (1927) and Bensley and Steen (1928) to be relatively insignificant, compared with the amount of glomerular excretion herein reported. Richards and Walker (1928), furthermore, restudying the elimination of dyes in the perfused frog's kidney, believe that the volume of glomerular excretion is sufficient to account for all of the dye which a frog's kidney excretes. A rough analogy to these two excretions might be the ability of the skin to eliminate urea and other substances in nephritis, which, however, are excreted in health by the preferential method of the glomeruli in the kidneys. Normally, the elimination by the skin is very small, probably accounted for by diffusion. In glomerular kidneys this perhaps is also the rule, but when the concentration of waste or foreign products in the blood become high enough, a certain amount—greater than in the normal state—escapes through the tubular epithelium, probably by a process not identical with what we know as secretion. In aglomerular kidneys, on the other hand, this process, being the only one at hand, must satisfy for the whole function; but being a less satisfactory method of elimination and being in a less developed group of cells, the process is slower than glomerular excretion. The fact that aglomerular kidneys in these fish excrete a larger volume of urine in proportion to their size than the fifty per cent glomerular kidneys, as was pointed out by Edwards and Condorelli (1928), lends support to the idea that the process of excretion through the tubular epithelium is of a type different from a secretion, as we ordinarily understand the term, and possibly is a diffusion. It also might indicate that the less specialized kidney might have limited powers of concentration.

Edwards and Marshall (1924) and Bensley and Steen (1928) have called attention to the studies of Nussbaum (1886) upon the living kidney of the Triton (salamander). Nussbaum injected a saturated solution of indigocarmine intravenously and reported that during the next twenty minutes, the urine in the bladder turned blue. He then went on to say that Bowman's capsule showed no dye, but that the tubules did contain dye. The second segment of the tubule showed minute pale granules of

pigment along the inner border of the epithelium and the fourth segment showed deep blue dye in the lumen. It is evident that Nussbaum missed the appearance of the dye in Bowman's capsule. It might have been due to the fact that he did not watch the glomeruli immediately after the intravenous injection of the dye, the time when it is easiest to see the dye solution in the capsule. In his description of the experiment he does not say just when he began his microscopic observations. The presence of dyes within Bowman's capsule in the frog's kidney has been conclusively shown by Richards and his co-workers, Edwards and Marshall, as well as by the workers in this laboratory.

Wearn (1922) and Wearn, Schmidt and Richards (1923–24) were the first to show that the tubules in the frog's kidney actually reabsorbed any substances, namely, sugar and sodium chloride, and they assumed the same for water, because the concentration of urea was greater in the bladder urine than in the glomerular capsular fluid. Bieter and Hirschfelder (1924) were the first to point out that dye solutions, being excreted by the living frog's kidney, were concentrated in the tubules on the ventral side of the kidney, which are the distal convolutions, and this undoubtedly is due to the reabsorption of water.

CONCLUSIONS

1. Injection of the glomeruli through the renal portal vein in the frog is a procedure easily accomplished, and, on an average, shows about a thirty per cent glomerular injection in the whole kidney, but in some cases it may mount to twice that figure.

2. The observation that phenolsulphonephthalein, when added to the renal portal blood or perfusate, is eliminated in the urine, as was first demonstrated by Rowntree and Geraghty, is here confirmed.

3. The mechanism of this elimination parallels the accessibility of the glomeruli to renal portal blood, and it is absent when the renal portal blood does not enter the glomeruli, as shown by simultaneous renal portal india ink injections.

4. The accessibility of the tubular capillaries to the renal portal blood flow appears to have no marked relation to the excretion of phenolsulphonephthalein injected as described above.

5. A new technique for the study of kidney function in the frog is described, by which substances injected into one or the other blood supply can then reach more or less of the glomeruli in the two halves of the kidney and thus result in varying secretions.

6. The frog's kidney submitted to this procedure is able to concentrate phenolsulphonephthalein at least for a period of fifteen to twenty minutes, thus indicating that the kidney is functioning in a manner close to normal during this time.

7. These results tend to confirm the conception of Nussbaum, that in the tubular capillaries, a complete mixing of renal arterial and renal portal venous blood occurs, and it is still further confirmed by Richards and his co-workers (1927–28).

8. These results also confirm Adami's criticism of Nussbaum's experiment; namely, that after ligation of the renal arteries in the frog, the glomeruli may still obtain blood, probably from the renal portal vein.

9. *Glomerular elimination of phenolsulphonephthalein in the frog's kidney appears to be the method of preferential excretion.*

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After this article had been written, a paper appeared by Oliver and Shevky (Journ. Exper. Med., 1929, l, 15) in which the authors have studied the excretion of phenolsulphonephthalein by the frog's kidney. Their technique consisted of perfusion with a modified Ringer's solution according to Höber (Klin. Wochenschr., 1927, vi, 671). They have obtained substantially similar results for the excretion of this dye as has been reported by the authors of this paper, thus further adding proof to the conception of the glomeruli of the frog's kidney being the *preferential route for the excretion of phenolsulphonephthalein*. Oliver and Shevky are apparently not familiar with our previous reports on this question, nor with the papers of Richards and Walker, 1927, on the injection of the glomeruli by way of the renal portal vein.

WORKING MECHANISMS FOR THE LIQUID AND GASEOUS INTAKE AND OUTPUT OF THE JACOBSON'S ORGAN

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Anatomical and histological studies by numerous investigators have demonstrated to a certainty the olfactory supply to the Jacobson's organs, and the presence therein of typical olfactory cells. The consensus of opinion is that these organs serve the animal in some olfactory capacity.

If they act as organs of smell, it is clear that this would necessitate some mechanism for "sucking" air or liquid into their rather inaccessible lumina, and another for ejecting it therefrom. This is nothing other than the fundamental principle underlying the olfactory function.

From his careful histological studies on the rabbit, dog, and guinea pig, Klein (1881–1882) was deeply impressed with the rich vascularity of the lateral walls of these organs, especially in the first and the last animals named. He describes it as resembling typical erectile or cavernous tissue, separated from the lumen of the organ by a thin, longitudinal layer of elastic tissue to which smooth muscle fibres are inserted. He made no suggestion concerning the functional significance of these special tissues.

Mihalkovics (1899) states that this muscle tissue served to dilate the lumen of the Jacobson's organ, and that the wide veins in the lateral wall act to compress it when ejecting its contents into the nasal cavity. He made no demonstrations on either the dead or living animal that these exchanges may actually occur. His experiments were of a simple nature, consisting in destroying Stenson's duct by thermal cautery in one cat and three rabbits. Afterward these animals took food and behaved generally as though nothing had happened to them. Therefore, Mihalkovics felt justified in concluding from his experiments that the Jacobson's organs were of no importance whatever to the animal, although he suggested that they might serve to distinguish wholesome from harmful substances.

In 1916 I carried out some experiments in which it was demonstrated that air could be expelled from the orifice of the Jacobson's organs in recently killed calves and rats by merely pressing a finger on them toward the orifice when the heads were submerged in water. This was contrary to Seydel's (1895) assertions that the Jacobson's organs never contain air

but only glandular secretion. The presence of air in these organs naturally suggested the importance of demonstrating its actual introduction and expulsion under as nearly normal conditions as possible.

In the meantime, Broman (1920) published an account in which he states that air seldom occurs in the Jacobson's organs but that liquid (secretion) is constantly streaming from them. His histological studies on the dog and the guinea pig led him to assert that the Jacobson's organ is both erectile and contractile, and to attribute to it a functional significance similar to that suggested by Mihalkovics. He did demonstrate, however, from some preserved lepidosaurians, that air and liquid came out of the Jacobson's organs when he pressed a probe on them and that some of the liquid was sucked back when the pressure was released. He performed a large number of experiments on horses, cattle, sheep and calves, in which he sprayed coloring matter into their nasal passages just previous to their being killed, and subsequently examined the Jacobson's organs to determine whether any of this stain had been sucked into their lumina. All of these results were negative. He therefore made no successful demonstrations on living animals that these erectile and contractile mechanisms actually perform the functions which he assumed.

While tracing out the blood supply of the Jacobson's organ, I made what seemed a significant observation. These vessels in the rat were filled with India ink injected from the aorta. Histological preparations of these specimens showed the presence of the ink and the more or less complete obliteration of the lumen of the Jacobson's organ. It was the latter observation that suggested that some factor of the circulation, such as the pulse or the blood pressure, might be the agency by which the contents of the Jacobson's organs could be more or less continually ejected throughout the life of the animal.

To determine such a possibility, the following investigations were planned and carried out on living animals. Rabbits were put under ether-urethane anesthesia; their upper incisors were extracted; the upper lip was slit and the left nostril trimmed away sufficiently to expose the orifice of the left Jacobson's organ; a tracheotomy was performed so as to remove confusing respiratory air currents from the nasal passages; the right carotid artery was cannulated and connected with a standard mercurial manometer so that the arterial blood pressure during the experimentation could be recorded; the left external jugular vein was cannulated for the introduction of pressor substances, such as adrenalin. A binocular microscope was used to observe the orifice of the Jacobson's organ following the intravenous injection of adrenalin. Due to the minute orifice of the Jacobson's organ, it was found that accurate observations could be made only by means of the binocular microscope.

Probably the most difficult step in the foregoing procedure was that

concerned with the exposure of the orifice of the Jacobson's organ. Hemorrhage was continually confronted. Besides the extraction of the teeth, a portion of the alveolar process supporting them had to be carefully trimmed away before the orifice could be distinctly seen. In trimming away the mucous membrane of this region of the nasal passages, every care had to be taken to avoid injury to the orifice. Usually a bristle was inserted in the orifice during observations, so that its location could be definitely known. In some cases where the secretion in the Jacobson's organ seemed insufficient, its lumen was filled with Ringer's warm-blooded solution previous to the administration of the adrenalin. This operation also had to be done with the greatest care in order to avoid hemorrhage from the Jacobson's organ itself. These injections were made with a 2 cc. hypodermic syringe, using a no. 26 gauge Luer needle.

Thus far six rabbits, including both males and females, have given positive demonstrations. That is, they have shown that a bubble of gas or a

TABLE 1

NUMBER OF POSITIVE DEMONSTRATIONS	MATERIAL EXPELLED	SIZE OF ADRENALIN DOSE (1:50,000 DILUTION)	MEAN CAROTID PRESSURE AT TIME OF EXPULSION OF GAS OR LIQUID
		cc.	mm. Hg
21	Liquid	$\frac{1}{4}$–$1\frac{1}{2}$	110–146
3	Liquid	$\frac{3}{4}$	70–94–104
7	Liquid	$\frac{3}{4}$–1	*
4	Air	$\frac{3}{4}$	68–80–94–110

* Exact point of expulsion not indicated on records.

drop of liquid (secretion or saline solution) appears at the orifice of the exposed Jacobson's organ when the effect of the adrenalin elevated the mean arterial pressure. This usually occurred when the mean pressure was above 94 mm. Hg. The normal mean carotid pressure for the rabbit, under experimental conditions, lies between 90 and 105 mm. Hg (Macleod, 1926). While most of the demonstrations were made for pressures above normal, some of them occurred when they were within normal limits. Thirty-five positive demonstrations have been made for the expulsion of either gas or liquid following the intravenous injection of adrenalin, as shown in table 1. These data would seem to leave no doubt as to the possibility of the blood pressure acting as the agency for effecting the removal of the contents of the Jacobson's organs. Whether it is the normal mechanism which operates during the life of the animal remains to be demonstrated. Whether higher pressures than the normal are usually required to expel the gas or liquid is a subject that is being further investigated. In one instance a drop of liquid (secretion) failed to be expelled

by a blood pressure of 90 mm. Hg, whereas pressures higher than normal immediately preceding and following resulted in positive demonstrations.

Recently eight successful demonstrations have been made showing the ability of the Jacobson's organs to "suck" liquid into themselves. Five of these observations were made on a male, and the remainder on a female rabbit while they were under ether-urethane anesthesia. In each of these demonstrations the drop of liquid (secretion) had been brought to the mouth of the Jacobson's organ by pressures elevated by means of adrenalin. Continued observation of the drop of liquid until the blood pressure is restored to "normal" shows that it is drawn back into the lumen of the organ as the pressure falls. The following table will show the pressure at which the drop of liquid appeared at the orifice, and that at which it was "sucked" into the lumen:

PRESSURE AT WHICH DROP OF LIQUID APPEARED AT THE ORIFICE	DOSE OF ADRENALIN (1:50,000 DILUTION)	PRESSURE AT WHICH THE DROP OF LIQUID RECEDED
mm. Hg	*cc.*	*mm.* Hg
114	½	100
130	1	84
132	1½	80
120	1½	100
110	½	56

Both Broman and Mihalkovics have assumed that the smooth muscle which is to be found between the lumen of the Jacobson's organ and the lateral wall of the Jacobson's cartilage is the mechanism for effecting the intake of air or secretion into the organ. The Jacobson's cartilage or cartilaginous capsule which more or less completely surrounds the Jacobson's organ is, according to Broman, an important part of the pumping mechanism in that (as he states) "it prevents the external soft parts from collapsing the organ." It seems to me that this cartilaginous capsule may have a twofold function. In the first place, it may act similar to the thoracic wall in respiration, as a provision against collapse when its internal pressure is lowered following the expulsion of its contents, either gaseous or liquid. At this point in the working of the pumping mechanism the specially located layer of elastic tissue, first mentioned by Klein (1881) and found in the writer's histological preparations stained in orcein, could come into play and restore the lumen of the Jacobson's organ to its normal size, and at the same time produce a condition of lowered internal pressure that would serve to "suck" liquid or air into the organ. It would seem that the importance of this special layer of elastic tissue has not been emphasized sufficiently as an essential part of the pumping mechanism. In the second place, the cartilaginous capsule (Jacobson's cartilage) might serve to facilitate a rise in internal pressure by preventing the

expansion of the Jacobson's organ at the time this pressure is being developed, thereby favoring the more complete ejection of its contents. In this way Jacobson's cartilage would facilitate both the introduction of fresh odoriferous material and its removal, once the latter has served its purpose; in its presence, the elastic tissue layer could more effectively "draw" this material into the lumen of the Jacobson's organ, and the blood pressure eject it.

While the demonstrations of the introduction of liquid into the lumen of the Jacobson's organ, and its expulsion therefrom, do not prove that these organs are olfactory in function, they nevertheless give us one more line of evidence supporting such an hypothesis.

The writer wishes to acknowledge his indebtedness to Dr. G. H. Parker, of Harvard University, under whom this problem was started, for stimulating suggestions, given in the early stages of this research. He wishes also to express his deep gratitude to Dr. Leonard B. Nice, of Ohio State University for significant suggestions and criticisms in the course of the recent work.

SUMMARY

1. Air and liquid may be forced out of the Jacobson's organs of rabbits under anesthesia, when their blood pressure is elevated by the pressor effect of adrenalin, as shown by 35 successful demonstrations.

2. Drops of liquid (secretion) are drawn into the Jacobson's organs of rabbits when the blood pressure recedes following the disappearance of the effect of the adrenalin.

3. The results of this research provide another line of evidence in support of the olfactory hypothesis for the Jacobson's organs.

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THE EFFECT OF HUNGER ON THE EMPTYING TIME OF THE STOMACH

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In studying the effect of various pathological processes on gastric emptying time, the factor of polyphagia or excessive hunger, secondary to intestinal indigestion or diarrhea, has been a complicating factor in the interpretation of the results.

The physiological literature on the effect of hunger or fasting on the emptying time of the stomach gives the general impression that hunger decreases the gastric emptying time. However, the only work bearing directly on the subject is that of Haudek and Stigler (1910), who found that the stomach empties faster when the food is eaten with hunger than without. Their data are too meager and haphazard to be conclusive, however. Cannon and Washburn (1912) cited this evidence to show that the stomach is in greater tonus during hunger, which is now an established fact. The stomach of the infant is known to manifest marked tonus and to empty rapidly (Carlson, 1916). The tonus of the stomach in the adult is proportional up to a maximum, with the length of starvation (Carlson, 1916) and more food or greater volume is required to inhibit strong hunger contractions than weak. Exposure to cold, mild exercise, diabetes, phloridzin glycosuria, feeding thyroid extract, loss of hair in the dog, and hemorrhage increase hunger contractions. In these conditions which require more food to meet the normal metabolic requirements of the body, one might teleologically argue that the stomach should empty faster in order to increase the rate at which food is supplied to the intestine for absorption. Also, gastric digestion is probably facilitated by an increase in appetite which increases the appetite secretion of gastric juice. Therefore, the evidence strongly supports the view that hunger decreases the gastric emptying time, but since only a single investigation of this possibility has been carried out, and since it is a factor that has a bearing on any study of the effect of various procedures on gastric emptying time, we decided to obtain direct evidence on the question.

METHODS. Men and dogs were used in these experiments.

The dogs used were gentle and were trained to lie quietly without being

disturbed by the fluoroscopic examination, which procedure was used to ascertain the gastric emptying time. The dogs were fed the usual stock diet at approximately the same hour every day except on the days when they were given no food and on the days when the gastric emptying time was to be determined. The barium test meal consisted of 125 grams of Swift's Silver Fox Food, 100 cc. of milk and 35 grams of barium sulphate. The fluoroscopic examination was in every instance of short duration so as to avoid any effect that exposure of the stomach and intestines to x-rays might have on the emptying of the stomach.

The "control" or normal emptying time for the test meal was determined in the dogs twenty-four hours after the ingestion of the last "stock meal."

TABLE 1

Showing the effect of hunger on emptying of the stomach in dogs

DOG	24 HOURS AFTER LAST MEAL		48 HOURS AFTER LAST MEAL		72 HOURS AFTER LAST MEAL		96 HOURS AFTER LAST MEAL	
	Number of tests	Average emptying time	Number of tests	Decrease in emptying time	Number of tests	Decrease in emptying time	Number of tests	Decrease in emptying time
				minutes		*minutes*		*minutes*
P1	3	5 hr., 17 min.	1	42	2	72	2	65
B2	3	5 hr., 12 min.	3	40	2	74	4	63
S3	4	5 hr., 7 min.	2	29	3	40	2	64
R4	4	4 hr., 10 min.	5	32	3	57	1*	30
S5	4	4 hr., 49 min.	2	29	5	52	3	66
W6	4	4 hr., 48 min.	2	33	5	55	3	70
B7	4	4 hr., 28 min.	2	23	5	41	3	55
Average decrease			17	32	25	56	18	57

* In one other test the emptying was not decreased. This dog was nervous and not as well trained as the others. Hunger increased his restlessness.

To determine the effect of hunger on the emptying of the test meal, it was given 48, 72 and 96 hours after the ingestion of the last meal. Between each test the dog was placed on the stock diet for several days in order to prevent loss of weight due to too much starvation.

The students reported for their test meal, which consisted of 120 grams of barium sulphate with 300 cc. of water at 37°C. approximately six hours after the ingestion of the last meal, which we call their control emptying time, and again twenty-four hours after the ingestion of the test meal.

Water was allowed ad libitum except during the time of the fluoroscopic observations. Only dogs that ate the test meal voluntarily were used.

RESULTS. *On dogs.* The results are recorded in table 1. In all dogs and in every test but one, hunger decreased the emptying time. It is to

be especially noted that the average decrease in the emptying time caused by 72 and 96 hours of starvation is the same, and an examination of the figures for each dog indicates that a maximum decrease is reached at 72 hours (dogs 1 and 2) and that in the other dogs the decrease from the 72nd to the 96th hour is small.

On men. Medical students were studied. The tests were not repeated. Of the seventeen, thirteen showed a decrease; three showed no change; and in one the emptying time was prolonged 30 minutes. One student

TABLE 2

Showing effect of hunger on emptying of the stomach in man

STUDENTS	RESULT	REMARKS
22 hours after control test meal		
Wh.	Shortened 60 minutes	Lower border of stomach in pelvis
Hut.	Shortened 30 minutes	
Mc.	Shortened 60 minutes	Lower border of stomach in pelvis
Her.	Shortened 30 minutes	
24 hours after control test meal		
Ca.	Shortened 15 minutes	Lower border of stomach in pelvis
Si.	Shortened 40 minutes	
As.	Shortened 60 minutes	
Ro.	Shortened 55 minutes	Lower border of stomach in pelvis
Ba.	Shortened 40 minutes	
Jo.	Shortened 40 minutes	
We.	No change	
Pa.	No change	Lower border of stomach in pelvis
Da.	Shortened 75 minutes	
Wa.	Shortened 30 minutes	
Hol	No change	
Tr.	Prolonged 30 minutes	Lower border of stomach in pelvis
Od*	Shortened 15 minutes	

* Student Od continued the fast to 54 hours. On taking the test meal he was nauseated and the emptying time was prolonged 30 minutes.

continued the fast to 54 hours, became nauseated on ingesting the meal and showed an increase of 30 minutes in his emptying time.

It is to be noted that the results were more variable in the men than in the dogs.

DISCUSSION. It is quite evident from these results on man and dog that up to a certain point hunger or fasting decreases the emptying time of the stomach, which confirms the observations of Haudek and Stigler (1910) and the general impression and the a priori opinion of a number of writers. The fact that after 72 hours of fasting the decrease becomes less and

variable in different dogs indicates that the optimum effect that increased tone of the stomach (due to hunger) has on its emptying time has been reached. This may be due not only to a limited capacity of the gastric musculature to increase its tone, but to inhibitory factors such as nausea, weakness, and restlessness which are associated with fasting in some cases.

These results also demonstrate that the factor of hunger must be considered in interpreting the effect of any procedure on the emptying of the stomach. For example, Lorenzi (1922) studied the effect of thyroid extract feeding on gastric emptying time and found the everage time to be about two and one-quarter hours less than the average normal before the thyroid feeding. We know that up to a certain point thyroid feeding increases appetite and causes polyphagia. Also Yesko (1928) and Fauley and Ivy (1929) found that pancreatectomy and ligation of the pancreatic ducts decreases the emptying time of the stomach and in these conditions there is a polyphagia.

CONCLUSION

Evidence is presented which shows that hunger or fasting causes the stomach to empty faster in both dog and man.

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STUDIES ON THE KNEE JERK

VIII. The Effects of Acutely Raised Intracranial and Intraspinal Pressures upon the Knee Jerk

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These experiments were undertaken in the hope of finding an early prognostic sign in cases of raised intracranial pressure following trauma. It was thought that a gross change in the patellar jerk might prove to be such a sign.

Very little appears in the literature concerning the absence or diminution of tendon reflexes in states of acutely increased intracranial pressure. Oppenheim (1) states that "an accumulation of cerebrospinal fluid, resulting in an increased pressure, could affect the posterior roots of the lumbar portion of the spinal cord even to the arresting of the conduction. As a result, degenerative changes may occur in those portions of the cord which serve as arcs." Strong sensory irritation of the posterior roots, as by compression, may depress the reflex movements according to Lapinski. Discussing chronic increased intracranial pressure, especially that due to hydrocephalus and tumors of the posterior fossa, Purves-Stewart says, "Thus these reflexes are lost in the final stages of increased intracranial pressure, especially in hydrocephalus and in tumors of the posterior fossa. This may be due to a curious degeneration of the posterior spinal roots which sometimes occurs in such conditions" (2).

A clinical observation upon the prognostic significance of the reflexes was made by Wilensky (3) in an analysis of 75 cases of skull fracture. He reached the conclusion that although changes in the reflexes were the most common evidence of neurologic disturbance, these changes had no definite bearing in the total clinical picture, especially from the therapeutic standpoint. Alterations of the reflexes were varied, consisting of equal or unequal responses and of diminution or exaggeration on one or both sides. Often the reflexes were absent soon after the accident, but soon returned, and then showed the variations just noted. Abnormal reflexes were frequently present. When the changes in the reflexes existed alone, aside from any focal lesions of the higher centers, the patients usually recovered when treated conservatively. Where indications of focal lesions were

present, the state of the reflexes was apparently incidental and unimportant.

It will appear from the evidence which we are about to present that we cannot agree with these conclusions of Wilensky's. Our experiments tend to show that the absence of the knee jerk may be a sign of considerable prognostic significance when the intracranial pressure is raised acutely. This discrepancy between his clinical and our experimental findings indicates that further clinical study of the problem is desirable.

METHODS. The knee jerk was the tendon reflex selected for experimental study because it is not only commonly tested clinically, but also because it can be accurately measured in the laboratory. The apparatus used for recording the knee jerk was that devised by Johnson (4). By means of it uniform blows of a suitable strength were delivered to the right patellar tendon at intervals of seven seconds. The excursion of the leg was recorded by a system of levers upon a slowly revolving smoked drum. Respiration, carotid blood pressure, time and intracranial pressures were recorded as well. For reasons of mechanical convenience, the intracranial pressure was recorded separately and therefore does not appear upon the published tracings.

The experimental animals were dogs, deeply narcotized by the injection into the upper peritoneal cavity of 250 to 300 mgm. of sodium barbital per kilogram, according to the method of Johnson (5). In a few instances paraldehyde, 1.5 cc. per kilogram by mouth was used, but produced less satisfactory experimental conditions.

The intracranial pressure was raised by a modification of Cushing's method (7). Through a midline incision in the scalp, the bone of the right parietal region was laid bare and a small opening was trephined therein. An accurately fitting brass cannula was screwed into the hole, and was connected by means of a T-tube with a mercury manometer and with a pressure bottle filled with 0.9 per cent sodium chloride solution. A hand bulb raised the pressure in the bottle and forced the solution into the cranial cavity.

Similarly in some of our experiments, we inserted a cannula into a trephine hole in the spinal column in the upper lumbar region. The cord was transected either through the opening or else immediately above it. Bleeding was checked by packing with cotton, which also effectively blocked off the portion of the spinal canal below the transection from that above. Thus we were able to observe the effects of direct pressure upon the lumbar and sacral cord alone. In all instances the pressure was brought rather quickly to the desired level, was maintained as nearly as possible at that level for a given length of time, and was then suddenly released.

It must be stated at this time that the dura exposed by trephining was freely incised in most of these experiments. When we began to work with

the transected cord, we at first left the dura intact, because we were afraid that the salt solution entering the subdural spaces under pressure might leak out at the level of the transection. However, we found that considerably higher pressures than usual were required to produce the same symptoms in an animal when the dura was intact than when it had been freely opened. Therefore we later incised the dura in all instances, and found that the cotton packing prevented leakage at the level of the transection in a quite satisfactory manner. Cushing has pointed out the importance of incising the dura when raising the intracranial pressure experimentally. He has shown that the fluid pressure applied outside the dura acts locally as a foreign body might, whereas, after the dura has been opened, there is an even distribution of pressure within the cranial cavity, no matter whether the point of application of the pressure be over the cerebrum, the cerebellum, or the spinal cord.

Experimental studies of increased intracranial pressure have been numerous. For a clear and comprehensive discussion of the symptoms the reader is referred to two articles by Cushing (7), (8). We propose to limit our discussion, in so far as is possible, to the new findings revealed by our experiments.

RESULTS. Seventeen dogs were used in these experiments, and of these, 15 produced useful records. The intracranial pressure was raised a total of 132 times, the intraspinal pressure, 31 times on these 15 animals.

In complete accord with the work of other experimenters we found that marked respiratory and blood pressure changes typical of acute intracranial compression manifested themselves when the intracranial pressure approached the level of the arterial blood pressure. These findings are charted in figure 1, A and B. On this chart the solid portions indicate the percentage of experiments where marked effects were produced; that is, 1, extremely irregular respiration, usually with periods of cessation of breathing; 2, blood pressure changes of 30 mm. Hg or more; 3, complete abolition of the knee jerk. Cross hatched portions indicate milder effects: 1, slight to moderate respiratory changes; 2, variations in blood pressure less than 30 mm. Hg, and 3, diminished height of the jerk. White spaces indicate the percentage of experiments in which raising the intracranial pressure produced no effect. The degree of the intracranial compression is expressed in percentage of the carotid blood pressure. It is easily observed that *the percentage of marked effects, both respiratory and circulatory increases as the intracranial pressure approaches the level of the arterial pressure.* At this level most of the effects observed were marked.

A. EFFECTS OF RAISED INTRACRANIAL PRESSURE ON THE KNEE JERK. *I. Cord intact.* 1. As the intracranial pressure approached the level of the carotid blood pressure there was either a considerable diminution in the amplitude of the knee jerk, or else a complete abolition of it (fig. 1 C).

Whether the jerk was diminished in height or abolished depended upon the reaction of the individual animal. In some dogs we were unable to abolish the jerk, no matter how high the intracranial pressure applied, until failure of the vasomotor center and rapid decline of the blood pressure caused its abolition through asphyxia. However, at pressures approaching or exceeding the arterial blood pressure, the knee jerk was usually diminished to such a point where it could probably not be elicited by the usual clinical means. To this extent the relationship of the magnitude of the intracranial tension to abolition of the knee jerk as charted in figure 1 is misleading.

2. The jerk usually returned promptly after the intracranial pressure was released unless the compression had been maintained for a considerable length of time after respiratory movements had ceased, when the return of the knee jerk was observed to be gradual or long delayed.

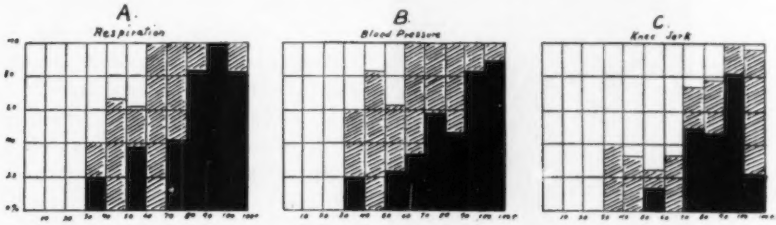

Fig. 1. Abscissae: Intracranial pressures expressed in per cent of arterial pressure. Black areas: Percentage of marked effects. Cross hatched areas: Percentage of slight to moderate effects. White areas: No effects.

Dixon and Halliburton (8) found the spinal centers to be deeply depressed, and the last to recover in their experiments on cerebral compression. This may be the case when the pressures used are very high (400 mm. Hg for 6 to 8 minutes), as in their experiments. We seldom used pressures of 200 mm. Hg applied for 1 to 4 minutes, and found that after compression of this order, the knee jerk usually returned with surprising promptness, the exceptions occurring only after there had been considerable asphyxia of the knee jerk center, due to prolonged application of the pressure and a long period of cessation of respiration. In our experience it was the usual thing for the knee jerk to reappear before the return of respiratory movements or simultaneously with them.

3. The diminution or abolition of the knee jerk was sudden in 70 per cent of the cases in which it occurred. A sudden diminution or abolition of the jerk was frequently the first manifestation (an often almost instantaneous one) of increased intracranial pressure, occurring before any respiratory or blood pressure changes had had time to manifest themselves (fig. 2).

4. A period of primary stimulation of short duration, as evidenced by an increase in the amplitude of the jerk, often preceded the abolition (44 per cent of the cases in which diminution or abolition occurred). Figure 4, D, E and F.

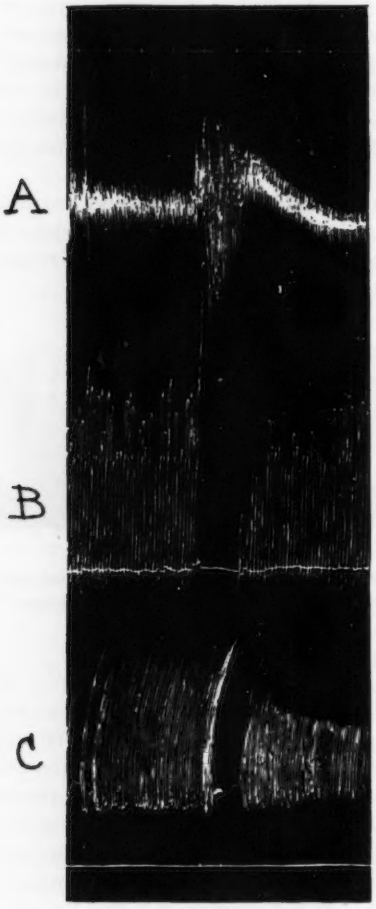

Fig. 2. Showing the sudden and complete inhibition of the knee jerk on raising the intracranial pressure. A = blood pressure record. B = Knee jerk record. C = Respiratory record.

II. After transection of the cord. After transverse section of the spinal cord in the upper lumbar region, and blocking off of the spinal canal, all effects upon the knee jerk were abolished. On raising the intracranial pressure the respiratory and blood pressure effects occurred as usual, but the knee jerk remained unaffected, unless it finally succumbed to asphyxia when the respiratory and vasomotor centers failed. Figure 3, A and B.

B. EFFECTS UPON THE KNEE JERK OF RAISING THE INTRASPINAL PRESSURE UPON THE ISOLATED LUMBAR AND SACRAL CORD. After transection of the cord in the upper lumbar region and blocking off of the canal, raising the pressure within this isolated portion of the canal resulted in no respiratory or blood pressure changes. The effects on the knee jerk were uniform and striking. There was quite regularly (66 per cent) a short period of primary stimulation lasting about 30 seconds, during which the height of the knee jerk was often enormously exaggerated. Then there occurred an abolition, usually sudden, of the jerk. Upon releasing the pressure the knee jerk returned, the promptness and rapidity of the return depending on the degree of the intraspinal pressure applied and the length of time during which it was maintained. Figure 3, C and D.

DISCUSSION. There is no doubt that the abolition of the knee jerk is

due to the anemia caused by the increased pressure within the cerebrospinal cavity and the resulting asphyxia of the knee jerk center in the lumbar cord. Additional evidence for this view is presented in figure 4, E and F, which is one of a number of similar records which we have obtained in the course of our experiments. Here, it will be observed, that upon raising the intracranial pressure, the knee jerk was first abolished for a very short time.

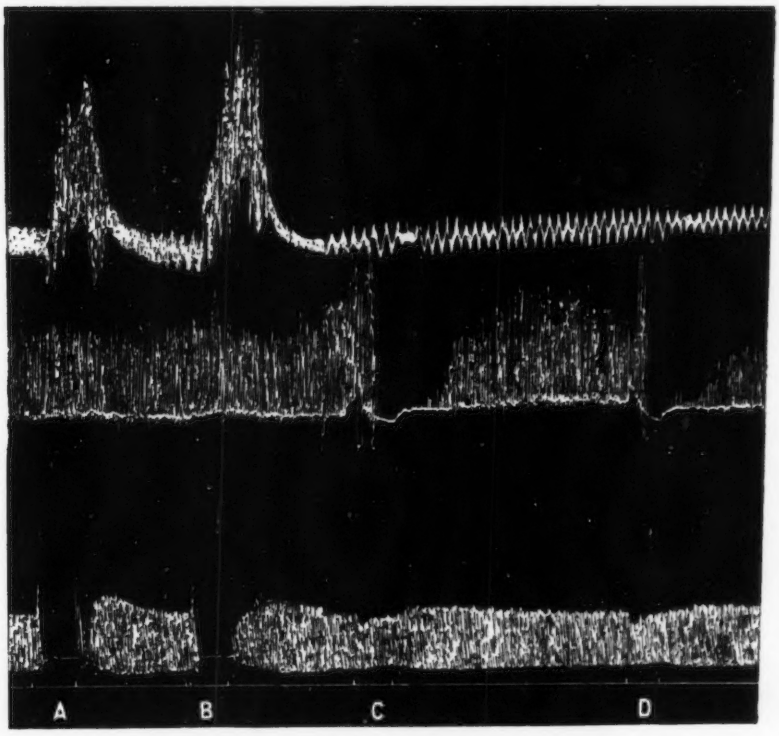

Fig. 3. A and B. Effects of intracranial pressure after transection of the lumbar cord. C and D. Effects of pressure raised upon the lumbar cord after transection. Note primary increase followed by sudden and complete abolition of the knee jerk.

As the vasomotor center responded and the blood pressure rose above the intracranial tension, respiratory movements returned, and at the same time the knee jerk again appeared, due presumably to restoration of oxygenated blood through the medulla and spinal cord. The same cycle was repeated with successive asphyxial rise in blood pressure until the intracranial tension was restored to normal.

In figure 5 B other asphyxial effects are illustrated. The cord has been transected and the spinal canal blocked off. Raising the intracranial pressure causes an almost immediate cessation of respiration and an enormous asphyxial rise in blood pressure. The knee jerk is not affected until the asphyxia has lasted for some time. Then the height of the jerk begins to diminish and continues to do so even after the intracranial pressure is released. However, artificial respiration administered at D has the effect of promptly restoring the knee jerk to more than its former level. Thereafter very shallow respirations were sufficient to maintain the jerk

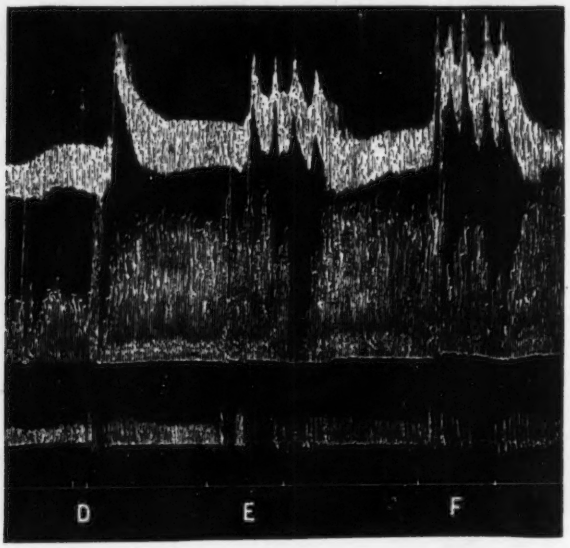

Fig. 4. D, E and F. Effects of intracranial pressures higher than the arterial pressure upon the knee jerk.

at a somewhat lower level than normal. Figure 5 C shows the knee jerk in the same animal abolished by simple asphyxia (clamping off the trachea).

Figure 6, one of two records obtained upon a single animal, is very interesting. In this case, after the lumbar cord had been transected the abdominal aorta was tied off as high as possible in the abdomen in an attempt to cut off the circulation to the knee jerk center. As a result we were able to produce the phenomena which we had observed when we raised the pressure directly upon the isolated lumbar cord, but these effects appeared slowly. There were first, a prolonged period of stimulation with an increase in the height of the knee jerk; later, a diminution in height and

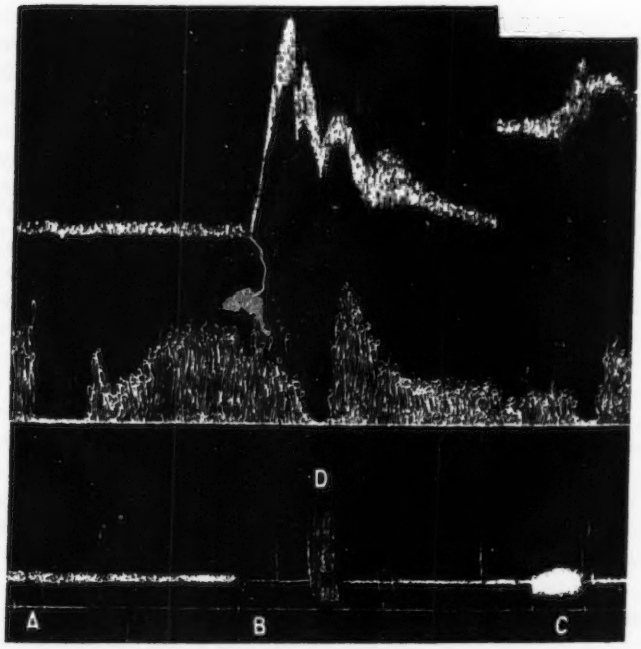

Fig. 5. A = Increased intraspinal pressure after transection of the lumbar cord. B = Increased intracranial pressure after transection of the lumbar cord. C = Asphyxia with abolition of the knee jerk. D = Artificial respiration.

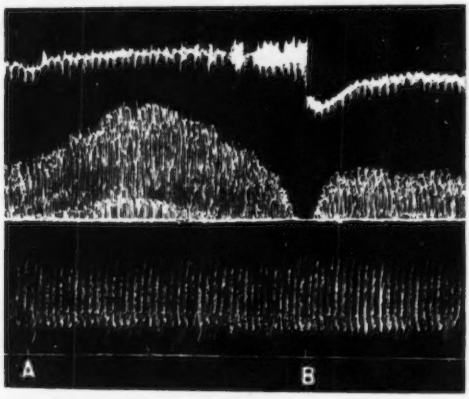

Fig. 6. Effect upon the knee jerk of tying off the abdominal aorta after transection of the lumbar cord. A = Ligature applied. B = Ligature released.

finally, abolition of the jerk. Upon releasing the ligature about the aorta, the reflex returned to about the normal level. These are isolated instances, but they all tend to show that the knee jerk is abolished on account of asphyxia of the centers in the cord.

A problem which is more difficult of solution is the question why the knee jerk is often so promptly abolished in experiments where the intracranial pressure is raised while the cord is still intact. In some instances the knee jerk may continue normally for several jerks, that is to say, for 20 to 40 seconds, and may then be suddenly abolished. These results appear comparable to abolition of the jerk by raising the pressure directly upon the lumbar cord, in which case there are several normal or exaggerated responses before the reflex disappears. We have stated that we believe these results to be due to asphyxia. However, we have observed not infrequently that the knee jerk disappeared promptly when the intracranial pressure was raised, and before any respiratory or blood pressure changes had had time to occur (fig. 2). It is difficult to explain this finding unless we suppose that the knee jerk center in the lumbar cord is influenced by inhibitory fibers from the brain, and that these fibers are stimulated in some way by increased intracranial pressure. This sudden decrease or abolition is not seen when the cord has been severed prior to raising the intracranial pressure.

SUMMARY OF RESULTS. 1. Increasing the intracranial pressure to a point where it approaches the carotid arterial blood pressure leads to a fairly abrupt diminution in the height or abolition of the knee jerk preceded in some instances by a few jerks of increased amplitude.

2. If the cord has been cut above the knee jerk center, the knee jerk is not affected until the respiratory and vasomotor centers have been paralyzed. The abolition of the knee jerk then seen is due to asphyxia of the center, and perhaps also to inadequate circulation through the center.

3. Increase in the intraspinal pressure following transection of the cord above the knee jerk center, results in a temporary but marked increase in the amplitude of the knee jerk, followed by a complete abolition of the reflex. These results are attributed to cessation of the blood flow through the cord (asphyxia), for ligation of the abdominal aorta in the upper lumbar region gives similar results.

CONCLUSIONS

1. The prompt diminution of the knee jerk following marked increase in intracranial pressure, very often prior to the occurrence of any changes in the blood pressure and respiration, is attributed to asphyxial stimulation (through their cell bodies) of inhibitory fibers acting on the knee jerk center.

2. A sudden gross diminution in or complete abolition of the knee jerk

observed in clinical cases of raised intracranial pressure would, on the basis of these experiments, seem to denote that the intracranial pressure is mounting to a dangerous level. The diminution or abolition of the knee jerk may be of value in indicating the necessity of early operative interference.

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VII. STUDIES ON THE PANCREAS

An Attempt to Repeat the Mellanby Procedure for the Isolation and Purification of Secretin

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For more than two years Ivy, Kloster and Lueth (Laboratory of Physiology, Northwestern University) have been attempting to isolate and purify secretin. Although they have been able to obtain in a number of instances a highly purified product (1928), active in doses of 0.2 mgm. in a 30 pound dog, and free of vasodilatin, they have not been successful in discovering a procedure that is uniformly reliable. Mellanby (1928) has reported a procedure for the isolation and purification of secretin based on the principle of "bile salt" precipitation, which yields, according to his report, a highly purified secretin. Because of the apparent directness and simplicity of his procedure and of the high degree of purity of his product, it was decided to try out his method.

Procedure and experiments. In all of our experiments Mellanby's procedure was followed to the letter with the exception of the source of the crude "bile salt" which Mellanby obtained from an English manufacturer. We used Merck's technical sodium glycocholate, since it was found that this gave a better precipitate under the conditions of Mellanby's procedure, than sodium taurocholate, a mixture of both, or a crude bile salt preparation. This sodium glycocholate was the most satisfactory of any bile salt preparation we were able to obtain. In all of our experiments, using Merck's sodium glycocholate, we never failed to get a precipitate following the addition of the bile salt solution and acidification with glacial acetic acid. Almost without exception precipitates and filtrates resulted as described by Mellanby.

Prior to our attempts to repeat Mellanby's procedure, one of us (Ivy) attempted to use the principle of "bile salt" precipitation on an active highly purified preparation of secretin. Sufficient alcohol was added to the solution of secretin to make it a 20 per cent alcoholic solution. Sufficient bile salt in 10 per cent aqueous solution was added to the alcoholic secretin solution so as to have a 0.2 per cent concentration of bile salt. This was acidified to the extent of 0.2 per cent with glacial acetic acid.

A precipitate resulted on standing in the ice box. The resulting precipitate was active. This procedure was repeated on the same solution a second time, but the precipitate was inactive and the activity could not be recovered from the filtrate. The procedure was repeated on the same solution without the addition of alcohol with two positive results out of a number of trials. A number of attempts have been made to ascertain why the "bile salt" precipitation principle works on this particular solution sometimes, but not uniformly.

In order to describe our experiments and results using Mellanby's method throughout, we will at this point describe his procedure.

MELLANBY'S PROCEDURE. 1. The mucosa is scraped from a number of duodena. In these experiments pigs' duodena were used. After grinding with sand, 250 grams of mucosa are extracted with a liter of absolute alcohol for 30 minutes at room temperature. The mixture is filtered through a coarse filter paper (Chardin) giving a clear yellow filtrate.

2. This filtrate is distilled in vacuo to about 40 per cent of its volume, i.e., until the liquid becomes opalescent and starts to froth. The solution is made up to the original volume with distilled water. This causes partial aggregation of the colloidal soaps and fats contained in the extract.

3. The removal of fats and soaps is effected by means of $CaCl_2$ (0.01 N) at a temperature of about 20°C. For this purpose 1 cc. of $CaCl_2$(N) is added to each 100 cc. of the slightly warmed fluid from 2. Within a few minutes the whole of the fats and soaps aggregate and form a well-marked precipitate which may be quickly filtered by a coarse filter paper.

4. The clear filtrate from 3 is then cooled in an ice chest. Two cubic centimeters of 10 per cent bile salt (commercial sodium tauroglycocholate) are added to each 100 cc. of the filtrate. After complete mixing, acetic acid to the extent of 0.2 per cent is added. A small precipitate is produced on adding bile salt, but this precipitate is increased by the addition of the acetic acid. The precipitated bile acids adsorb secretin and carry it out of solution. Flocculation and settling of the precipitate occur more rapidly at low temperatures. Therefore, after addition of the bile salt and acetic acid, the mixture is left for one or two hours in an ice chest.

5. The precipitate from 4 is obtained by centrifuging, and is taken up in a small volume of absolute alcohol. Some of the precipitate does not dissolve. This may be removed by spinning and rejected.

6. The alcoholic solution of secretin and bile acid is added to five volumes of acetone. After a short time a flocculent precipitate of secretin separates out and may be obtained as a compact mass by spinning in a high speed centrifuge. The precipitate is washed with acetone (twice) and dried with ether.

7. Further purification is obtained by dissolving the precipitate from 6 in a small volume of water in which this secretin is freely soluble. Acetic acid is added to the solution to the extent of 0.1 per cent. The secretin is precipitated and may be obtained by spinning, and drying whilst in the centrifuge tube with acetone and ether. The final product is very active and has all the characteristic properties which are described in the following pages:

OUR EXPERIMENTS. *1st experiment:* Five kilos of mucosa were scraped from the first six feet of the intestines of one hundred recently killed hogs

and extracted with 20 liters of absolute alcohol. This extract is carried through the above steps.

The absolute alcohol solution obtained in 5 is added to 5 volumes of acetone. The secretin should separate out as a flocculent precipitate. A slight amount of solid separated out after the mixture stood overnight in the ice chest. This was collected and assayed for secretin. The assay showed 12 threshold doses, free of dilatin. A threshold dose is the dose sufficient to augment definitely the continuous secretion of the pancreas. The absolute alcohol-acetone mixture from which this solid had separated out was evaporated to dryness, the solid thus obtained was washed with acetone and dried with ether and then dissolved in H_2O. This solution was assayed for secretin and showed none to be present. The residue not soluble in absolute alcohol in 5 was dried with ether, then shaken with water. This aqueous extract yielded about 100 threshold doses of secretin. The original liquor in which the bile salt precipitation had been carried out was evaporated to dryness and the solid taken up in water. This water extract yielded no secretin.

Although we observed the reactions described by Mellanby, we obtained a yield of only 12 doses and were able to account for only 112 doses beyond the "bile salt" precipitation step in the procedure. The yield should have been at least 5400 doses as indicated by the following experiment. From 100 guts extracted with 0.4 per cent HCl and the solid precipitated with NaCl, we obtained 9400 doses. From the residue of the 100 guts after absolute alcohol extraction, we obtained 4000 doses; therefore 5400 doses should have gone into the alcoholic extract which was precipitated with "bile salt." Ivy, Kloster and Lueth have found that only about one-half to two-thirds of the secretin in the gut extractable with 0.4 per cent HCl is soluble in 90 to 95 per cent alcohol.

2nd experiment: Not believing absolute alcohol to be necessary to extract the scraped-off mucosa, we decided to use 95 per cent alcohol for the extraction in this experiment. The mucosa from 100 guts (first six feet) was extracted with 95 per cent alcohol (1 liter for each 250 grams of mucosa).

The solid obtained in 6 of the Mellanby procedure from an original 95 per cent alcohol extract of pigs' duodena was washed twice with acetone, dried with ether and then dissolved in H_2O. The assay for secretin showed approximately 2500 doses. The amount of solid determined per dose was 0.3 mgm. It was attempted to purify this further by step 7 in Mellanby's procedure. The solid which came out of the solution on the addition of acetic acid was collected and dried. It was not readily soluble in water, and the solution did not contain much activity. The aqueous solution of secretin to which the acetic acid had been added and which now was $\frac{1}{10}$ per cent in acetic acid still assayed for secretin, although half of the activity was lost in the precipitation. This secretin could not be recovered from the precipitate.

The "bile salt" precipitation worked this time giving a yield of 2500 doses, the theoretical yield being at least 5000 doses. Step 7 of Mellanby's procedure failed to work.

3rd experiment. In this experiment another absolute alcohol extraction was made. The mucosa from 100 guts was extracted with absolute alcohol and the extract carried through step 6 of Mellanby's procedure. The aqueous solution of the solid obtained in step 6 was assayed for activity and showed a total of 250 threshold doses, about double the number obtained in the first trial. The theoretical yield is 5000 doses, approximately.

4th experiment: In our next experiment, we repeated the 95 per cent alcohol extraction in the hope of securing enough crude secretin to carry out further purification experiments. The mucosa from 300 guts was extracted with 95 per cent alcohol and the extract carried through to step 6. This time the secretin yield was very low, a total of 1500 doses being obtained against a theoretical yield of 15,000 doses. No attempt was made to purify this material further by the acetic acid precipitate.

5th experiment: We repeated the Mellanby procedure using the mucosa from ten dogs' duodena. No secretin was obtained in this experiment. On repeating the experiment a second time a total of 10 out of a theoretical 400 doses was obtained.

DISCUSSION. Mellanby's method for the isolation of secretin is based upon three observations: 1, secretin may be extracted from the intestinal mucosa by absolute alcohol; 2, precipitated bile acids adsorb secretin from solution; and 3, dilute acid precipitates secretin from aqueous solution.

In step 3 of the procedure the fats and soaps are removed by 0.01 N $CaCl_2$ at a temperature of 20°C. Mellanby mentions the possibility that at this stage there is always some loss of secretin since $CaCl_2$ precipitates the bile salts which are always present to some extent in the alcoholic extract of the duodenal mucosa, and that these precipitated bile salts carry some secretin out of solution. We investigated this phase of the problem too, but were unable to discover any secretin in the solid which was filtered off.

In our work we have found that 95 per cent alcohol is serviceable for the Mellanby procedure as well as absolute alcohol. Our results show that the principle of "bile salt" precipitation as used in the Mellanby procedure was not uniformly reliable and in no instance gave the approximate theoretical yield. Dilute acetic acid precipitation did not prove at all satisfactory in our hands.

We feel that Mellanby's procedure has considerable promise because when it works it yields a highly purified product almost entirely free of "dilatin." It is unfortunate that we do not know more about the factors concerned in governing the "bile salt" precipitation. Our attempts to ascertain such

factors, which are unnecessary to mention in this paper, have met with failure.

SUMMARY

The isolation of secretin as described by Mellanby proved to be unsatisfactory in our hands because of the unreliability or lack of uniformity of results with the "bile salt" precipitation procedure. In default of more exact knowledge regarding the conditions governing the adsorption of secretin by "bile acids," we were unable to predict a yield with certainty.

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ELECTRON EQUILIBRIA IN BIOLOGICAL SYSTEMS

III. The Measurement of Electrochemical Potentials By Means of a Vacuum-tube Static Voltmeter Which Eliminates Minute Polarization Currents

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In the first two papers of this series (Voegtlin and DeEds, 1; DeEds, Rosenthal and Voegtlin, 2) we have presented a method for the continuous measurement of electrochemical potentials and methods for the simultaneous automatic recording of electrochemical potential and muscular contraction. In these publications no stress was laid upon the question of whether or not the potentials measured are absolute potentials. We were concerned only with the experimental observation that rhythmic functional changes are accompanied by rhythmical potential changes and that induced functional changes are characterized by potential changes superimposed upon the normal potential changes. The question of the absolute value of the basic potential with reference to that of a standard cell and the absolute magnitude of the rhythmic change in potential constitutes a separate problem fraught with many difficulties. Some of the necessary steps toward the solution of this question constitute the subject matter of this paper.

In the previous publications it was stated that under the specified operating conditions no current flows in the grid circuit of the first tube of the vacuum tube voltmeter. "This is a circumstance of the greatest importance for the measurement of potentials in biological systems, for it meets the requirement of measuring potential variations without the withdrawal of currents from the systems of small capacity[1] under investigation." The reasons for stating that no current flows in the grid circuit may be found in the first publication. If, aside from theoretical grounds, previously pub-

[1] Wherever the term capacity is used in this paper with reference to the measurement of a chemical system, the term refers of course to capacity in the physicochemical sense, according to the equation

Chemical energy = Chemical potential × Chemical capacity

The capacity is the quantity factor. Just as the chemical potential can be conveniently measured in volts, so the capacity can be measured in Faraday's—the quantity of electricity carried in electrolysis by one gram equivalent.

lished, it can be experimentally established that no current flows, then it follows at once that the potentials reported are true basic values and that the rhythmic fluctuations have a true order of magnitude.

A simple and conclusive experiment furnished the answer to the above question. We succeeded in making micro platinum electrodes by Taylor's method (3) and inserting them into amoeba without any apparent effect upon the organism other than a localization of its movements. So long as the micro-electrode was not connected to the vacuum-tube voltmeter the amoeba appeared normal, but upon connecting to the voltmeter a minute bubble was seen to form at the micro electrode. This bubble grew in size until it caused the amoeba to burst. This experiment demonstrated conclusively that a small current was flowing in the grid circuit of the first tube and that, because of the small surface area of the micro-electrode, the current density at the electrode was sufficient to liberate a gas, either hydrogen or oxygen depending upon the direction of current flow. Although it is true that a flow of current sufficient to prevent measurements on a system having a capacity as low as an amoeba did not prevent the attainment of true values on systems of larger capacity, yet we were confronted with the necessity of measuring the current flowing, and, if possible, modifying the equipment so as to prevent it, for we wish to measure potentials in amoeba and other single cells eventually. It is evident that so long as a small grid current flows it is likely that the potentials found in systems of small capacity are too high or too low depending upon the direction of flow of electrons in the small polarizing current of the grid circuit. It also seems likely that the magnitude of any fluctuations would be decreased by the polarization.

These few introductory remarks emphasize the need for measurement of *absolute values*, and this can only be done with a *static* measuring equipment. It is, therefore, the object of this paper to describe a 3-tube static voltmeter and its use, to compare the instrument with a quadrant electrometer, to describe biological and physical methods of detecting minute grid currents, and to show the relation of grid current to the value of the potential measured.

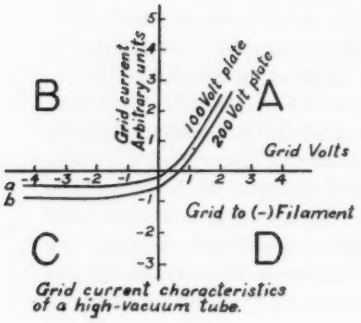
THE THREE TUBE STATIC VOLTMETER. In the present voltmeter a third tube has been added, for the simple reason that the operation of the first tube under conditions giving rise to a minimum current (of the order of 10^{-12} amperes) in the grid circuit of the first tube reduced the amplification. A third tube has given even greater amplification than existed in the former two tube apparatus. Before proceeding to a description of the new voltmeter it will be necessary to discuss the possible causes of current flow in the grid circuit.

It will be assumed that the reader is familiar with the construction of an ordinary three-electrode vacuum-tube, such as the UX 201-A, and with

the functions of filament, plate and grid. Since the magnitude of the charge on the grid, under conditions of constant filament current and plate voltage, determines the number of electrons landing on the plate and hence the value of the current, it is evident that a variation in the charge on the grid causes an amplified alteration in the value of the plate current. It is also evident that so long as the changes in the grid charge are superimposed upon a charge that is always more *negative* than the filament of the tube, only a very small current can flow in the grid circuit. If, however, the grid is not sufficiently negative relative to the filament appreciable current will flow in the grid circuit. Insufficient negative grid bias is therefore one cause of grid currents and results in a flow of electrons from the grid to the system being measured. Fortunately this source of difficulty is relatively easy to eliminate. Reference to quadrant A of figure 1 shows in arbitrary units the manner in which the magnitude of this current varies with the plate voltage and the positive charge of the grid. The second and most troublesome cause of current flow in the grid circuit is due to imperfect evacuation of the tube and consequent ionization of the residual gas and traces of mercury used in efforts to perfect the vacuum. In this case the direction of the electron flow in the grid circuit is from the system being measured to the grid. The magnitude of this current is dependent upon the plate voltage. The obvious remedy for this source of current flow in the grid circuit consists of the use of a very small plate voltage, a highly evacuated tube and a negative grid bias large enough to prevent current flow due to the first cause, but small enough to prevent "blocking of the tube," i.e., complete prevention of electrons landing on the plate. In other words, the first tube is operated under conditions which make it principally a pick up device and reduce its amplifying power. The second stage is a UX 240 tube used because of its high amplification power and the third stage is a UX 201-A tube used to restore the amplification lost by the mode of operating the first tube, and because it gives only a small distortion over the usual range of voltage change measured.

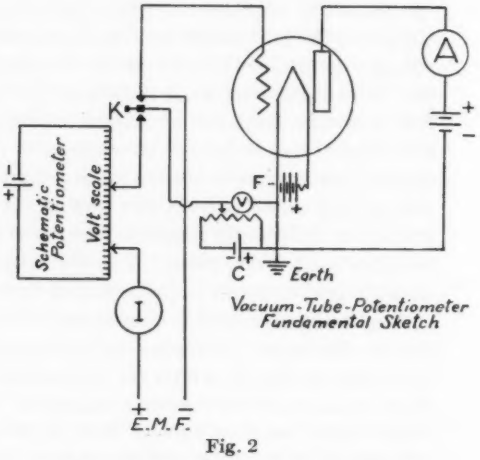
Aside from the fact that a third tube has been added, a biasing battery inserted between the negative pole, grid lead and the negative side of the filament, and that meters have been included to measure the plate voltages and plate currents, the voltmeter differs little from that described in the second paper. The connections for the all important first tube and potentiometer are shown in figure 2. Following the first tube are two stages of resistance coupled amplification with a rugged short period galvanometer in the output of the third tube. Figure 3 shows a detailed diagram of the whole voltmeter together with *approximate* values for resistances and voltage.

The reader may be puzzled why such a large plate resistance was used in the output of the first tube, the so-called "general utility" tube or 201-A.



Grid current characteristics of a high-vacuum tube.

Fig. 1



Vacuum-Tube-Potentiometer Fundamental Sketch

Fig. 2

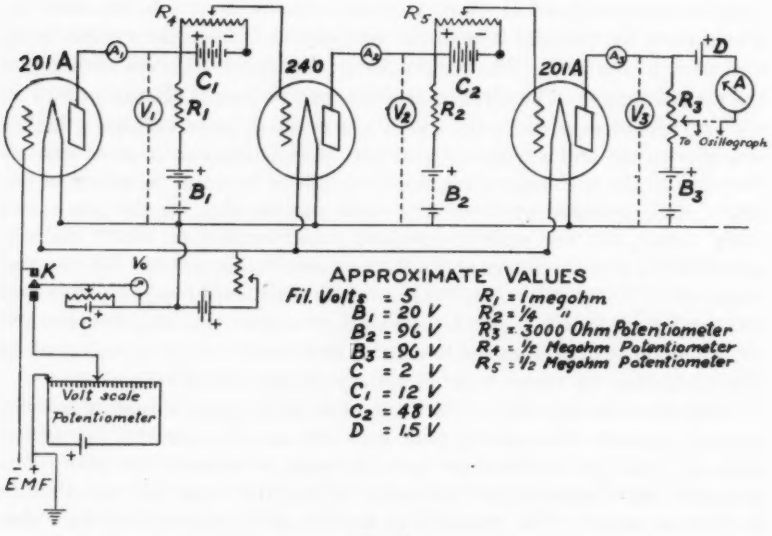


Fig. 3

This tube is not normally used in resistance coupled amplifiers. In our case, however, where only 10 or 15 volts were used on the plate of this tube the plate resistance jumps to a high value so the tube becomes essentially a "resistance" tube and a large plate resistor may be used.

Although a B battery of 20 volts is used on the plate of the first tube, the

actual voltage is $E = 20 - IR$, where I is the plate current and R is the megohm resistor in the plate circuit. In actual use I is about 5 micro amperes, so $E = 20 - 5 \times 10^{-6} \times 10^{6} = 15$ volts. The e.m.f. to be measured is balanced by means of a potentiometer so that the milliameter A in the plate circuit of the third vacuum tube (fig. 3) gives the same reading as when the zero key, K, is depressed. The voltage is then obtained from the potentiometer scale.

Some general recommendations concerning the equipment are: 1, the use of storage batteries throughout instead of dry batteries; 2, proper grounding of the tables on which the apparatus is assembled; 3, good insulation[2] by means of proper materials (sulphur, amberite) and wherever possible air lines for the high voltage wires; 4, operation of the first tube in a dry enclosed or evacuated vessel to prevent leakage over the base of the tube.

In practice the maximum voltage amplification that such an arrangement is capable of delivering is seldom utilized. To get the best results the indicator instrument resistance should match the resistance of the last tube. It is desirable to sacrifice the theoretical amplification of several hundred in order to obtain the advantage of an indicating instrument in the output of the last stage which is capable of standing heavy overloads and which has at the same time a short period of, say, $\frac{1}{3}$ second and portability. As used such an arrangement gave ordinarily 2 divisions on the indicating meter for 1 millivolt input. An oscillograph with a period of $\frac{1}{3}\frac{1}{0}\frac{1}{0}$ second may also be placed in the output of the last stage for automatic recording as was done in previous work (2).

If now for such an arrangement curves for the first tube are plotted with grid current against plate voltage B and grid voltage C relationships as in figure 4 are obtained. In measuring the grid currents it was necessary on account of the small magnitudes involved to use a galvanometer of the highest sensitivity, a Coblentz-Thompson iron clad astatic type.

As these curves show, an old tube (one which has been used a few months) gives a much smaller grid current than a new tube. As the voltage on the plate is lowered the grid current drops. The smallest currents we could reliably measure with the Thompson galvanometer were with 40 volts on the plate of the tube. With 20 volts on the plate of a selected old tube the currents were so small they could not be measured directly by any galvanometer.

MEASUREMENT AND ADJUSTMENT OF GRID CURRENT. The quest for satisfactory operating conditions of the voltmeter has been characterized

[2] With high atmospheric humidity it is impossible to prevent insulation leakage even by using the highest grade of insulation material. It is therefore necessary to provide apparatus for drying the air. This can be done by blowing the air over a radiator connected with a refrigeration plant.

by an ever increasing refinement in the method of measuring the magnitude of the grid current. At first a portable Leeds-Northrup galvanometer (sensitivity of 75 micro volts per millimeter with external damping resistance of 2000 ohms in series) was used and it became possible to adjust the amplifier so as to have a grid current of 2×10^{-8} amperes. Using a Leeds-Northrup galvanometer (sensitivity of 2 micro volts per millimeter with external critical damping resistance of 300 ohms in series) suspended from the ceiling the grid current was reduced to 2×10^{-9} amperes. Later a Thompson galvanometer was used and it was possible to demonstrate that the grid current was not more than 10^{-10} or 10^{-11} when careful adjustment of the first tube was made. At this point it is interesting to note that Elder and Wright (5) using a glass electrode and a single vacuum tube potentiometer found that the glass membrane showed polarization when the grid current was 7×10^{-9} amperes, but that this effect disappeared and correct pH values were obtained when the current was 2×10^{-9} amperes or less. We have also found that for the measurement of very poorly poised "redox" systems such as a m/100,000 solution of a 50/50 mixture of potassium ferro and potassium ferri cyanide or very poorly buffered pH systems, a grid current of 2×10^{-9} amperes does not interfere with the measurement of absolute values free of drift. But it always is difficult to measure living tissues, even when the grid current is too small to be detected by the Thompson galvanometer.

To make clear the difficulty involved, it will be necessary to digress briefly. Let us suppose we wish to measure a system of large capacity such as an ordinary dry cell, measuring 1.5 volts, and we do so with an ordinary potentiometer combined with a null point galvanometer. At first the tapping system is used as the potentiometer is adjusted to the point of no deflection on the galvanometer. When the adjustment has been made it will be found that the tapping key may be kept closed and the galvanometer needle will show no drift. This nicety of adjustment and absence of drift is due to two factors; first, a system of high capacity is so slightly altered by the small polarization produced by unbalance and its potential is so well maintained that a slight degree of unbalance in the potentiometer is easily detected and consequently adjustment is readily obtained. A small error in balancing at the null point results in so small a polarizing E M F that the system of *large* capacity is relatively unaffected. Suppose, however, we wish to measure a system showing an E M F of 1.5 volts but having an *extremely low* capacity. Because of the low capacity the polarizing current due to unbalance of the potentiometer alters the system and hence renders the balancing of the potentiometer a slow and more difficult process. The balance can not be obtained as accurately as in the first case, although the need for a perfect balance is greater. Consequently one can do no better than approach a perfect balance and any

attempt to keep the tapping key closed results in a drift of the galvanometer needle.

Since our problem was fundamentally one of reducing polarization currents to the point where no interference was encountered, when measuring extremely low capacity systems, it seemed worth while to compare the electrometer with the potentiometer-galvanometer method.

Figure 5 is a diagrammatic sketch of the simple quadrant electrometer and potentiometer hook-up. The needle is operated at 50 to 100 volts. To simplify shielding the potentiometer may be eliminated and the E M F connected directly to the electrometer if the exact potential is not required and the potential difference is 100 m.v. or less. The shielding necessary depends entirely on local conditions.

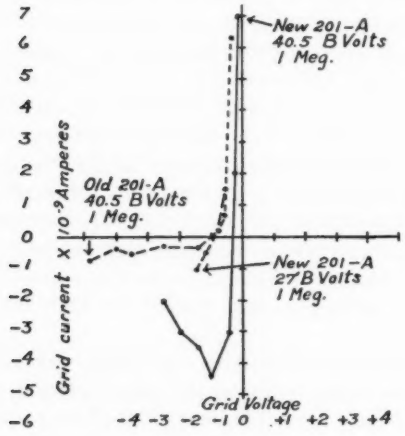


Fig. 4

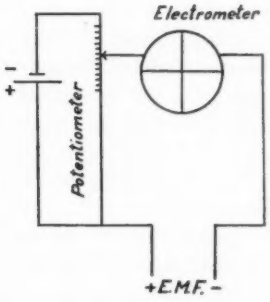


Fig. 5

The electrometer gives a negligible leakage of current due to insulation causes, is sensitive, and requires a current to operate it only sufficient to change the potential of its quadrants, which have a capacity of around 10 micro-micro farads = 10^{-11} farads. In measuring a potential difference of one hundred millivolts a quantity of electricity determined from the relation $C = \frac{Q}{V}$ must flow. C is in farads, Q in coulombs and V in volts. Now the number of coulombs determine the amount of electrolysis and polarization. This gives for the electrometer 10^{-12} coulomb of electricity, which in more familiar terms would be 10^{-12} amperes flowing for 1 second. Comparing such a current with that occurring in another very widely used combination, the ordinary galvanometer-potentiometer system, the superiority of the electrometer is obvious.

Using a galvanometer of sensitivity such that one scale division corresponds to a current of 10^{-9} amperes and assuming that the potentiometer is balanced within $\frac{1}{10}$ division for a period of 10 minutes or 600 seconds we see that the number of coulombs is (seconds $\times$ current in amperes) $600 \times (10^{-9} \times \frac{1}{10}) = 6 \times 10^{-8}$. Dividing by the number of coulombs for the electrometer gives $\frac{6 \times 10^{-8}}{10^{-12}} = 6 \times 10^{4}$ or 60,000.

That is, the galvanometer-potentiometer method would cause 60,000 times as much polarization and electrolysis as the electrometer.

The chief defect of the electrometer is its sluggishness, the period being from 1 to 10 seconds so that rapidly fluctuating potentials do not correctly register. Also it cannot conveniently be used with a recording oscillograph.

The vacuum tube static voltmeter offered a solution to the problem for it provided a fast reading instrument possessing the advantages of the electrometer. Such an equipment, when properly adjusted by means of a Thompson galvanometer, solved the difficulties for many low capacity systems when the grid current was 2×10^{-9} amperes or less, the criterion of satisfaction being a steady null point of the galvanometer with the tapping key closed. The small electrochemical capacity of the frog gastrocnemius, however, required a much smaller grid current, too small to be measured by means of a Thompson galvanometer. It, therefore, occurred to us that the frog gastrocnemius muscle in situ offered a *biological* means of detecting a minute grid current and might be used as a means of obtaining an adjustment of the amplifier so as to render it a truly static instrument.

The first tube with the grid floating or insulated, of course, meets the requirement that no current either enters or leaves the grid, excluding the insulation leaks over the tube base. After observing the plate current for this condition, the grid is now hooked up to the bias battery and the voltage adjusted until the plate current is the same as before. This voltage is known as the voltage for the floating grid and is the value which should be obtained if the external grid circuit were free from insulation leakage. In other words, this is the "true" grid value. In practise, however, the insulation leaks which usually occur are balanced by so adjusting the grid current that it exactly neutralizes in direction and amount the insulation currents.

If a perfect tube with no grid current could be obtained it would be a great improvement in case there were little or no external leakage. In case the external leakage is not zero, however, the advantages of a tube with no grid current would not amount to much.

When an attempt was made to get the potential difference against a calomel cell, using a blank platinum electrode inserted into the muscle in situ, difficulties arose if the grid was not maintained at the correct value.

A steady drift in the basic value of the muscle was at first obtained in our early measurements with the tube voltmeter even when no grid current could be detected with the Thompson galvanometer. Evidently the slight grid currents produced enough electrolysis in the muscle to steadily shift the potentials developed. It was early discovered that these shifts in value are greatly accelerated by using micro-electrodes, presumably due to the fact that with small electrodes for a given grid current the density of current is high. The density of current and hence polarization effects per unit area of electrode surface should be inversely proportional to the electrode area.

The fact that a muscle is so sensitive to the passage of current over a time of 15 minutes may be utilized as a method for the critical adjustment of the grid voltage. By obtaining values for the drift in potential differ-

TABLE 1

Comparative values

MATERIAL MEASURED	INSTRUMENT	VALUE IN VOLTS
Ringer's solution	G	0.044
	V	0.044
	E	0.044
Frog gastrocnemius muscle	G	Shifting
	V	0.0247
	E	0.0245
Glass electrode, Disodium phosphate-Potassium acid phthalate	G	None possible
	V	0.2744
	E	0.2750

G = galvanometer-potentiometer; V = vacuum-tube static voltmeter; E = electrometer-potentiometer.

ence with different grid voltages the curve of figure 6 was constructed so that the grid could be set at the critical voltage. When this was done the potential reading for the muscle remained constant and agreed exactly with the value obtained with the electrometer (see table 1).

It was determined that for this kind of a reading the potentiometer should be adjusted to within 20 or 30 millivolts of the true potential when taking the measurements.[3] If the value of the potential difference being measured did not fluctuate more than this amount there was no appreciable error involved. This implied that the grid voltage was correctly adjusted to within a few millivolts. Conversely, if the potentiometer was balanced the grid bias should be correct to within 30 millivolts.

[3] So far as the grid current is concerned the effect of an arbitrary change in potentiometer setting is the same as an equal voltage change in the grid bias battery adjustment.

The method finally adopted as being the quickest and most convenient for measuring the grid currents is in principle a very generally used and simple one. If through a known high resistance R a small unknown current I is passed, Ohm's law gives for the potential drop $E = IR$. Putting in the grid circuit a known high resistance, say 100 megohms, and reading the change in voltage E given by the vacuum tube equipment, we get directly the grid current from the above relation. We use for the resistance a thin glass membrane or "glass electrode" (fig. 7) having the same buffer solution on each side. Such cells are well known (6).

When the potentiometer key is up we have the situation as in figure 8. The only potential difference impressed between the filament and grid is that due to the C battery E_c, neglecting any result of the small grid current on this circuit. When the potentiometer key is down the circuit is as

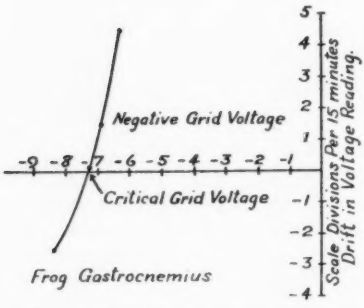

Negative Grid Voltage
Critical Grid Voltage
Frog Gastrocnemius
-9 -8 -7 -6 -5 -4 -3 -2 -1
5 4 3 2 1 -1 -2 -3 -4
Scale Divisions Per 15 minutes Drift in Voltage Reading.

Fig. 6. Numbers on abscissa are negative tenths of a volt.

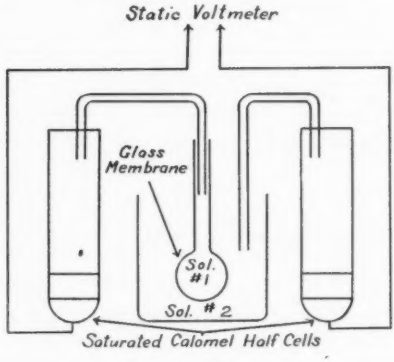


Fig. 7

shown in figure 9. The enormous resistance of the glass cell R causes a potential drop due to the flow of the grid current I_G, $I_G R = E_R$. To obtain the same voltage between grid and filament as in figure 8 a potentiometer voltage E_p must be inserted so that $E_p = E_R$. Usually the glass has a uni-directional voltage strain E_s of 0 to 50 millivolts which must be determined by reversing the polarity of the glass cell. With the glass cell turned one way we have

$$E_{p_1} = E_R + E_{\text{strain}}$$

while reversed, the conditions are

$$E_{p_2} = E_R - E_{\text{strain}}$$

from which

$$E_{\text{strain}} = \frac{E_{p_1} - E_{p_2}}{2}$$

Two such cells had resistances of 80 to 230 megohms respectively when measured with a sensitive galvanometer. Using in round numbers 200 megohms as the resistance of a cell we can write down the currents corresponding to different voltages read off on the potentiometer.

DEVIATION E	R	I (AMPERES)
millivolts	*ohms*	*amperes*
0.1	2×10^8	5×10^{-13}
1		5×10^{-12}
10		5×10^{-11}
100		5×10^{-10}

Now setting the grid bias voltage at different values gives different grid currents. A curve may be plotted using currents against voltage just as was done for the galvanometer. The point of zero grid current or the nearest thing thereto may be determined and the grid maintained at this value for the most exact readings (fig. 5).

We have made tests on several types of tubes—222, 199, 240, 201, 210—and conclude that the 201-A tube well aged to "clean up" the residual gases is as good as any tube available. It is probable that tungsten filament tubes are preferable to the oxide coated type on account of the large amount of occluded gases in the latter.

There is a great difference in the characteristics of apparently similar equipments of the same type. Thus one equipment with 201-A tube gave a critical grid value of -0.72 volt and another with a different tube gave a value of -1.72 volts. Moreover it has been found that equipments change in characteristics during the day, which we assume is due to the slow evaporation of occluded gas from the wall of the tube as it is heated up. Such tubes should be warmed up for several hours prior to the taking of actual readings. Changes in humidity also change the setting.

A description of the cause of the positive and negative grid currents may be found elaborated in radio treatises (7).

For the sake of emphasis it will be repeated that the current flowing when the grid is positive is due to the flow of electrons in the tube from the filament to the grid. When the grid is negative positive ions are attracted to it. These positive ions occur due to ionization of the traces of gas in a vacuum tube by collisions between the rapidly moving electrons and the gas molecules. Tubes of high evacuation should be used and the smallest plate voltage impressed consistent with the desired amplification, since under these conditions the number of molecules ionized will be diminished.

The essential feature of our work is the operation of the first tube at the critical point (fig. 10) or the point where the grid current-grid voltage curve crosses the zero current axis. To find this point requires individual cali-

bration of each tube used in the first stage as well as adjustment to this point prior to the performance of each experiment.

If a large fluctuating source of E M F is to be measured wherein the value changes over 100 millivolts it is best to operate the first tube on the flat part of the curve (fig. 1) rather than at the critical point. Under these conditions the grid is more negative than at the critical value. The necessity for this procedure is in preventing the grid from swinging to a positive value in which case large grid currents would result. But it should be borne in mind that the grid current resulting from operation on the flat part of the curve, while satisfactory for many systems, is too large for obtaining correct values with any system of very low electrochemical capacity.

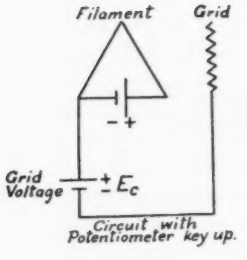


Fig. 8

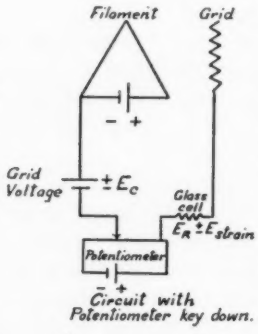


Fig. 9

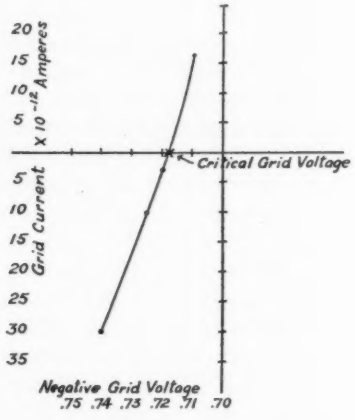


Fig. 10

It was a simple matter to verify that physiological salt solutions and oxidation-reduction systems, using a blank platinum electrode of fairly large surface, gave the same values on the electrometer and the vacuum tube voltmeter even when the first tube was off from the critical grid voltage adjustment by a few tenths of a volt.

Use of glass electrodes. When using glass electrodes the behavior of the vacuum tube voltmeter is as described. To secure the correct value of the system under measurement the grid of the first tube should be operated at the critical point. When this is done close agreement is obtained for the H-ion concentration (pH) of solutions. As an example of this we give two measurements: *example 1:* A Weston cell measured by the Bureau of Standards at 1.0189 volts was put in series with a glass cell which had no strain and having the same solution on both sides. With equipment I

having a grid voltage of -0.78 the values with the glass cell measured both forward and backward were 1.0189 and 1.0189 $\pm$ 0.0003 volt.

With equipment 2 having a grid voltage of -2.0 the values were also 1.0189 $\pm$ 0.0003 volt.

Example 2: Using an $\frac{M}{5}$ di-sodium phosphate solution on the outside of the glass cell and a potassium acidphthalate solution on the inside and a glass electrode with a strain of 1.6 mv., the electrometer-potentiometer set gave 275 mv. and the vacuum tube set gave 274.4 mv., a satisfactory check.

It should be stated here that we have made no attempt at physical measurements of greater precision.

It is likely from preliminary experiments that very small, comparatively thick electrodes of glass will give better values on the electrometer than with the vacuum tube equipment.

It was determined that the two methods for the critical adjustment of the grid bias, the glass electrode and muscle methods give identical values. The glass electrode method is much faster and can be conveniently used as a standardizing device by having a permanent glass electrode calomel cell equipment at hand.[4]

CONCLUSIONS

A vacuum tube voltmeter is described, which, when adjusted by means of a glass electrode becomes a static instrument. This instrument gives values agreeing with the electrometer on all systems measured except on thick glass electrode systems where the electrometer may be superior. The voltmeter has the advantage that high speed changes may be detected on feeding the output of the last tube into a recording oscillograph or simply using a fast moving indicating meter. The importance of the elimination of minute polarization currents in accurate measurements of physical and biological systems of small electrochemical capacity is demonstrated.

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[4] With the vacuum tube voltmeter adjusted to the critical grid bias it was easily shown that the essential conclusion of the correlation of potential change to muscular contraction and relaxation in the first paper of this series is still true.

THE NATURE OF THE FOODSTUFFS OXIDIZED TO PROVIDE ENERGY IN MUSCULAR EXERCISE

III. The Utilization of the "Waste Heat" of Metabolism in Muscular Exercise

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PART I. FAT, CARBOHYDRATE AND PROTEIN. In the earlier communications of this series (Rapport and Ralli, 1928a, b) evidence was advanced to show that in muscular exercise of mild character and short duration the respiratory quotient of the excess metabolism of exercise and recovery showed no greater tendency to approach unity than did the quotient at rest. Marsh (1928) has recently published observations which clearly showed that the excess R.Q. was influenced by the diet. As a rule, the excess R.Q.s were higher than those in the resting state, but the divergence was not usually large, and the discrepancy it seems to me could be in part at least explained by the difficulty of calculating the excess R.Q. with nice exactness when the subject is not yet in the post-absorptive state. Miss Marsh's independently obtained results in a general way coincided with ours. Experiments by Wilson, Levine, Rivkin and Berliner (1927) in very mild exercise by children, though the metabolism of rest periods after exercise was not obtained, have also shown a correspondence between resting and excess R.Q.s.

Our evidence was at variance with that of A. V. Hill and his collaborators, particularly Furusawa (1926), whose results indicated in mild exercise an excess R.Q. of unity which tended to fall as the exercise became more severe. Recently, however, Best, Furusawa and Ridout have published a paper (1929), in which the previous observations of Furusawa were contradicted, the excess R.Q. in mild exercise being practically that of the resting metabolism. As there is now no dispute as to fact in these conditions, the observation may be accepted as established.

Our own experiments led us to the conclusion that not only carbohydrate but fat could provide energy for muscular exercise, the amount of each that happened to be used in a particular case depending upon the proportions in which these substances were presented in available form to the muscles. Best, Furusawa and Ridout, in referring to the work of

Rapport and Ralli, say that "these studies show that the quotient of the excess metabolism of very mild exercise in dogs is much below unity." Since our R.Q.s *did* sometimes approximate unity on a high carbohydrate diet, this statement is not entirely accurate, nor does it do justice to a fact that seemed to us of considerable importance, namely, the striking coincidence of the resting and the excess quotients, whether the former were high or low. This coincidence caused us to conclude that whatever the oxidative processes in muscle, there was from this viewpoint no empirical reason for believing that they were not essentially similar to those of other tissues. In other words, the exercising animal shows no greater tendency to oxidize carbohydrate than does the resting animal.

Although Best, Furusawa, and Ridout obtained R.Q.s similar to ours in mild exercise, they found quotients of unity or above (as high as 1.7) when the exercise was severe. (Confirmation of these findings would be desirable, in view of the fact that they are contrary to those of previous investigators.) As a result of their observations they advance the theory that in mild exercise there is a conversion of fat to carbohydrate, and that in severe exercise this process cannot keep pace with the demand for fuel, resulting, presumably, in a drawing on carbohydrate stores, for they state: "the increased combustion of carbohydrate may then overshadow the effect due to the contemporary manufacturing process." This theory is difficult of acceptance partly because of the assumption that carbohydrate is the sole fuel of muscular contraction; though even granting the assumption, it is hard to see why, if sugar alone be burned to supply energy in exercise, the carbohydrate stores rather than fat should not be drawn on first. It is also not amiss again to point out that this theory, like others, involves the illogicality of regarding only unity, out of all possible R.Q.s, as a true combustion quotient.

If, as I believe, fat can be used directly as a source of energy in muscular exercise, it follows that the oxidative metabolism of contracting muscle is non-specific, that is, that the energy for the endothermic processes of recovery can be furnished by the oxidation of more than one foodstuff. It seemed pertinent then to inquire whether indeed the process was *at all* specific, or whether *any* exothermic reaction could furnish the required energy.

On this point some data exist. It is clear that protein plays a negligible rôle in the process. Moreover, Rubner (1910) found that when a man performed a given amount of mechanical work after ingesting a large amount of meat, the extra heat due to the food was summated with that due to the work. In other words, the exothermic reactions involved in the specific dynamic action of protein were unable to furnish free energy for the performance of work. This was confirmed by Anderson and Lusk (1917), who found that alanin behaved like protein in this respect.

In the course of their experiments, however, Anderson and Lusk also made the important observation that the energy of the specific dynamic action of glucose could be used for the performance of work, thus introducing another clean-cut distinction between the metabolism of glucose and that of protein and amino acids.

Benedict and Murschhauser (1915) performed experiments on the energy transformations during walking, and state that "the general impression derived from the comparison of all the experiments was that the heat per unit of work was practically independent of food." They thought this impression should be slightly modified when diets containing large amounts of protein were considered, for then "a slightly higher heat-output per unit of work may be expected."

In view of our earlier observations relating to the use of fat in muscular contraction, I was interested to see whether, in addition to this, the extra energy released as a result of the specific dynamic action of fat could also be used for the performance of work. The experiments here reported indicate that this extra energy of fat metabolism, which at rest appears as "waste heat," is, during exercise and recovery, utilized by the body for the performance of work. Incidentally, I have confirmed the observation of Anderson and Lusk in regard to glucose. Thus these two foodstuffs behave similarly in this respect, and a sharp distinction is to be drawn between them and protein.

EXPERIMENTAL PROCEDURE. The experiments were performed on a single well trained dog, who was given the customary standard maintenance diet of about 60 per cent carbohydrate, 25 per cent fat, and 15 per cent protein. The animal was caused to run on a horizontal treadmill introduced into a closed system for obtaining the gaseous exchange as previously described. The exercise consisted of 10 minutes' running at about 1.4 miles per hour. Employing the term "work" loosely to indicate the mass of the animal moved through horizontal distance, the "work" done was approximately, to use Benedict and Murschhauser's phrase, 3,000 horizontal kilogrammeters. Experiments during the post-absorptive state, at least 18 hours after the last food, were alternated with experiments after the ingestion of either fat, glucose, or meat. Most of these latter experiments followed the ingestion of fat. The exercise was preceded and followed by periods during which the resting metabolism was obtained, and the excess metabolism of exercise and recovery was calculated upon the difference between the exercise and recovery gaseous exchange, and that of the average of the pre- and post-exercise resting periods, as had been done in the previous work.

Four alcohol checks were carried out during the course of the experiments, with resulting respiratory quotients of 0.662, 0.665, 0.670, 0.669, showing a maximum error of the apparatus of less than 0.8 per cent during this period of time.

EXPERIMENTAL RESULTS. 1. *Experiments in the post-absorptive state.* Seven experiments were performed in the post-absorptive state (see table 1). The average resting heat production was 12.7 cals., certain observations being well over 13 cals. This is some 10 per cent higher than the basal heat production of the same dog previously, as reported by Rapport and Beard (1928). The difference is undoubtedly to be chiefly accounted for by the fact that in the present experiments the animal had to lie on a necessarily rather narrow treadmill, and complete relaxation was difficult. This

TABLE 1 (Series 1)

*Experiments in the post-absorptive state**

DATE (1928)	RESTING METABOLISM†			WORK DONE‡	EXCESS METABOLISM OF EXERCISE AND RECOVERY					CALORIES REQUIRED TO MOVE 1 KG. 1 M.	
					½ hour period§		1 hour period§				
	O_2	Calories	Non-protein R.Q.		O_2	Calories	O_2	Calories	R.Q.	Based on excess metabolism of ½ hour period§	Based on excess metabolism of 1 hour period§
	gm. per hr.			*kgm.*	*gm.*		*gm.*				
June 5	3.52	11.78	0.81	2990	2.20	7.58	2.48	8.43	0.77	2.54	2.82
June 11	3.82	12.77	0.83	2979	2.06	7.31	1.98	6.98	0.88	2.45	2.34
June 16	3.75	12.36	0.74	2946	1.91	6.44	2.24	7.32	0.73	2.19	2.49
June 19	4.14	13.68	0.76	2929	2.21	7.37	2.69	8.74	0.70	2.52	2.98
June 24	4.01	13.18	0.74	2986	1.92	6.58	2.41	7.94	0.77	2.20	2.66
June 27	3.89	12.95	0.79	2868	1.84	6.36	2.08	7.07	0.80	2.22	2.47
September 2	3.86	12.67	0.73	2845	2.37	7.72	2.60	8.40	0.71	2.71	2.95
Average	3.85	12.7	0.77	2935	2.04	7.05	2.35	7.84	0.76	2.40	2.67

* On most of these days, the diet of the preceding day consisted only of fat given for experimental purposes.

† Average of metabolism before exercise and after recovery.

‡ Running on a horizontal treadmill for 10 minutes at about 1.4 miles per hour. The term "work" is used loosely.

§ Refers to the duration of the period following the beginning of exercise.

is reflected in the "resting heat" variations, which were greater than those usually observed when the animal can lie comfortably relaxed.

The respiratory quotient at rest was low, even lower on the average than that observed after the ingestion of fat. This apparently anomalous R.Q. was due simply to the fact that the "post-absorptive" experiments in most cases followed an experiment on the previous day in which fat was ingested. On such days no further food was given, and the animal therefore was subsisting, during the "post-absorptive" experiments, largely on her own fat and on that ingested the morning before.

On consulting table 1, it will be observed that the excess metabolism of exercise and recovery is calculated in two ways—on a "½ hour period" and on a "1 hour period." This requires explanation, since it has a bearing on the interpretation of results. The "½ hour period" means that after the 10 minutes' exercise had been completed, an additional 20 minutes of rest were permitted before the period was closed and the gaseous exchange determined. The reasons for this were discussed in a previous paper (Rapport and Ralli, 1928a). During the succeeding ½ hour period, the evidence indicates that this particular animal still had a small oxygen debt. Recovery was invariably complete at the end of this second period. In order to calculate exactly the amount of extra energy required by the dog to do the specified work, both of these periods should be included as a basis for calculation. In the "post-absorptive" experiments this is the method of choice, and was adopted in the previous researches of this series. Thus it is found that when calculated on the ½ hour period, the animal apparently required on the average 2.40 cals. to move 1 kilogram 1 meter; while when calculated on the full 1 hour period, the dog needed 2.67 cals. for the performance of this amount of work. It is clear that the latter is the true figure.

Nevertheless, for the purpose of comparison with the excess metabolism of exercise after the ingestion of food, it is better to use the gaseous exchange of the first ½ hour period after exercise as a standard. The reason for this is as follows. Although this period does not include all the excess heat production due to the exercise, yet recovery is apparently completed before the end of the succeeding ½ hour period. During a portion of the latter period, therefore, the animal no longer requiring energy for the recovery from exercise, the specific dynamic action of the ingested foodstuff now comes into play uninfluenced by the recovery factor, and an extra heat production is obtained which confuses the point at issue, namely, whether the extra heat of specific dynamic action can be used in muscular work. This complicating factor is shown by a comparison of table 1 and table 2, the difference between the heat production of these two experimental series being greater when the full hour period is used than when only the ½ hour period is used. Since work is done at approximately the same rate both in the "post-absorptive" experiments and in those where food is previously ingested, the first ½ hour period of exercise and recovery, even though it does not represent the entire excess energy exchange of exercise, can nevertheless be used for purposes of comparison, and this has been done in the present experiments.

The animal in the post-absorptive state actually required 2.67 cals. to move 1 kilo of body weight through 1 meter. This number of calories is equivalent to 1.14 kgm. The animal used by Anderson and Lusk (1917) required only 0.580 kgm. of energy to do this amount of work. Our

figure is the same as that obtained by Slowtzoff (1903). It is apparent, as Anderson and Lusk point out, that the variation in the efficiency of different animals is considerable. Moreover, from the evidence adduced by many workers, man, in horizontal walking, is more economical of energy than are dogs moving at the same speed.

As was observed in the previous work of Rapport and Ralli, the respiratory quotients of the resting metabolism and of the excess metabolism of exercise showed a close correspondence, their average being almost identical, and the maximum deviation being 0.05.

TABLE 2 (Series 1)

*Experiments after ingestion of fat**

DATE (1928)	RESTING METABOLISM†			WORK DONE‡	EXCESS METABOLISM OF EXERCISE AND RECOVERY‖					CALORIES REQUIRED TO MOVE 1 KG. 1 M.	
					½ hour period§		1 hour period§				
	O_2	Calories	R.Q.		O_2	Calories	O_2	Calories	R.Q.	Based on excess metabolism of ½ hour period§	Based on excess metabolism of 1 hour period§
	gm. per hr.			*kgm.*	*gm.*		*gm.*				
June 4	4.83	15.92	0.75	2913	2.13	7.24	2.27	7.63	0.90	2.48	2.62
June 12	3.99	13.53	0.86	3012	2.10	7.48	2.27	8.05	0.87	2.48	2.67
June 13	4.85	15.87	0.72	2879	2.55	9.11	3.29	10.96	0.80	3.16	3.81
June 15	4.75	15.64	0.76	2860	1.92	6.57	2.31	7.78	0.83	2.30	2.72
June 18	4.75	15.73	0.80	3080	1.89	6.67	2.43	8.38	0.89	2.17	2.72
June 21	4.51	15.04	0.82	2913	2.11	7.29	2.42	8.31	0.81	2.50	2.85
August 31	4.37	14.45	0.77	3136	2.45	8.24	2.67	8.96	0.80	2.63	2.86
September 1		15.15	0.75	3103	2.37	7.97	2.78	9.09	0.72	2.57	2.93
Average		15.17	0.78	2987	2.19	7.57	2.55	8.65	0.83	2.54	2.90

* 75 grams corn oil in alkaline emulsion 2 hours before exercise.

† See table 1.

‡ See table 1.

§ See table 1.

‖ Above the resting level in the post-absorptive state, for which see table 1.

2. *Experiments after the ingestion of fat.* Fat was given to the animal by stomach tube in the form of an emulsion of 75 grams of corn oil with 10 cc. of 1 per cent Na_2CO_3. A preliminary hour was followed by two half-hour resting periods, after which the stated exercise was performed (about 2 hours after the fat was ingested).

The average resting metabolism before and after exercise, in 8 experiments, was 15.2 cals. per hour as compared with 12.7 cals. when the animal had had no food, a specific dynamic action of 20 per cent (table 2).

Nevertheless, calculating the excess metabolism of exercise on the first ½ hour after the beginning of exercise, it will be seen that the dog required 2.54 cals. to move 1 kilo through 1 meter, as compared with 2.40 cals. in the post-absorptive state, an increase of only 5.8 per cent. To put it another way, almost a calorie of the "waste" energy must have been used for the purpose of exercise and recovery. (For the full hour period almost double this quantity has to be similarly accounted for.)

The evidence points clearly to the fact that the specific dynamic action of fat, which at rest is dissipated as waste heat, is, during muscular exercise and the recovery therefrom, used as a source of free energy.

TABLE 3 (Series 1)

DATE (1928)	RESTING METABOLISM†			WORK DONE‡	EXCESS METABOLISM OF EXERCISE AND RECOVERY¶¶					CALORIES REQUIRED TO MOVE 1 KG. 1 M.	
					½ hour period§		1 hour period§				
	O_2	Calories	R.Q.		O_2	Calories	O_2	Calories	R.Q.	Based on excess metabolism of ½ hour period§	Based on excess metabolism of 1 hour period§
Experiment after ingestion of glucose*											
	gm. per hr.			*kgm.*	*gm.*		*gm.*				
September 5	4.43	15.36	0.98	3032	2.01	7.38	2.31	8.77	1.02	2.38	2.89
Experiments after ingestion of meat											
June 23‖	5.28	17.81	0.88	2988	3.00	10.40	3.57	12.54	0.84	3.48	4.20
September 7¶	4.75	16.18	0.86	2954	2.89	9.99	3.40	11.71	0.78	3.38	3.96

* 40 grams 2 hours before exercise.
† See table 1
‡ See table 1.
§ See table 1.
‖ 200 grams beef heart 2 hours before exercise.
¶ 100 grams beef heart 2 hours before exercise.
¶¶ Above the resting level in the post-absorptive state.

The average respiratory quotient of the resting metabolism was 0.78; that of the excess metabolism 0.83. This difference I do not regard as significant, in view of the fact that after the ingestion of a foodstuff the R.Q. at rest changes from hour to hour, making the exact calculation of the excess R.Q. more difficult than in the post-absorptive state.

3. *Experiment after the ingestion of glucose.* Anderson and Lusk (1917) found that after glucose ingestion mechanical work may be performed at an expense of 5 per cent less energy than when no food is taken. I have

repeated their experiment (though the work done was considerably less than in the case of their animal), and have confirmed their observation of the abolition of the specific dynamic action of glucose during work (table 3). The dog was given, in one experiment, 40 grams of glucose about 2 hours before the exercise was performed. The average resting metabolism was 15.4 cals. per hour, indicating a specific dynamic action of 21 per cent. During exercise, however, practically the same number of calories were required to move 1 kilo 1 meter as when no food was given—2.38 as compared with 2.40. In the second half-hour after the exercise, when recovery had been completed, the specific dynamic action of the glucose again became evident, for the heat production was well above the basal level.

As in the case of fat, therefore, the "waste heat" of glucose metabolism ceases to be waste heat during exercise and recovery.

TABLE 4 (Series 1)

Heat production at rest and in exercise and recovery

	RESTING (PER HOUR)	DEVIATION FROM (A)	EXCESS IN EXERCISE AND RECOVERY (PER KGM. OF WORK)*	DEVIATION FROM (A)
	calories	*per cent*	*calories*	*per cent*
A. In the post-absorptive state	12.7		2.40	
B. After ingestion of fat	15.2	+19.7	2.54	+5.8
C. After ingestion of glucose	15.4	+21.3	2.38	−0.8
D. After ingestion of meat 100 gm.	16.2	+27.6	3.38	+40.8
D. After ingestion of meat 200 gm.	17.8	+40.1	3.48	+45.0

* Based on ½ hour period after beginning of exercise, for reasons discussed in the text.

4. *Experiments after the ingestion of meat.* Protein, however, presents an entirely different picture. Rubner (1910) found that when exercise was performed after the ingestion of meat by a man, the extra heat production due to the work and to the food were summated; and Anderson and Lusk (1917) made the same observation. I have performed two experiments of this nature (table 3), and have obtained similar results. Thus, after 100 grams of chopped beef heart, which raised the resting metabolism 28 per cent, the animal required 41 per cent more energy to perform work than in the post-absorptive state; and after 200 grams of meat, which gave a specific dynamic action of 40 per cent, 45 per cent more energy than when no food was given were needed to perform work. This complete summation is clear confirmation of the conclusions of the earlier observers.

For the convenience of the reader, a concise comparison of the heat production at rest and during exercise and recovery is introduced in table 4, the difference between carbohydrate and fat, on the one hand, and protein on the other, being manifest at a glance.

PART II. EPINEPHRIN AND TYROSINE. That epinephrin has a definite stimulating effect on the energy exchanges in the body is well established, although the nature of the stimulus is as obscure as is the specific dynamic action of the foodstuffs. On account of the mobilization of sugar that has been observed as a result of epinephrin injections, the possibility existed that the "calorigenic" action of the substance was simply the specific dynamic action of carbohydrate. The experiments of Boothby and Sandiford (1923), confirmed more recently by Soskin (1927), adequately disposed of this notion, which the present experiments, as will be hereafter shown, also indicate to be untenable. As to the locus of the stimulating effect, it is in dispute, the observations of Hunt and Bright (1926) suggesting that epinephrin has a *general* calorigenic action on tissue metabolism, while those of Soskin indicate that the effect is dependent upon the presence of the liver. The predominant foodstuff oxidized to provide the extra energy exchange seems to vary with circumstance. It is true that as a rule a rise in the R.Q. following epinephrin injection has been observed, indicating an increased combustion of carbohydrate, but under certain conditions, as for example in the experiments on obese people by Krantz and Means (1927), the predominant foodstuff may be fat, even though more carbohydrate may be utilized than before the administration of epinephrin.

It seems clear then that for aught we know to the contrary, epinephrin exerts a specific type of stimulus to metabolism, and the increased exothermic reactions that occur in the body as a result of its administration may from this viewpoint be considered as an entity. These reactions, in the resting condition of the animal, involve the production of "waste heat" just as does the specific dynamic action of the major foodstuffs. The question that I propose to consider, as in part I of this paper, is whether this "waste heat" can be utilized in muscular work. A paper by Scheuchzer (1928), working in Asher's laboratory, seems to indicate that it can. In rats, running on a treadmill, this investigator found that the increased metabolism of about 40 per cent induced by epinephrin injection was almost completely abolished in exercise, for the excess O_2 consumption in work after epinephrin was only 7 per cent greater than when no epinephrin had been administered. The author concludes that "unter dem Einflusz von Adrenalin die Arbeit Wesentlich ökonomischer geleistet wird." I am quite unable to confirm this result, for my experiments indicate that the excess O_2 consumption and heat production due to epinephrin and that due to exercise are completely summated.

It is perhaps unnecessary to add that this does not in the least contradict the conclusion of Cannon and his collaborators (1929) that adrenalin injections have a highly favorable effect on the maximal capacity of dogs to run. The fact that work is done at a greater expenditure of energy does not affect the possibility that epinephrin may increase the ability of muscles to con-

tract; and fatigue, as their paper points out, is not a function of exhaustion of the fuel supply.

EXPERIMENTAL PROCEDURE. The method of experimentation was similar to that in part I of this paper. As these experiments were carried out several months after the first series, they required validation by new alcohol checks. Three of the latter were performed, yielding respiratory quotients of 0.669, 0.668, and 0.659, respectively, the maximum error being 1.2 per cent. The urinary nitrogen was determined as usual, and indicated that there was no appreciable difference between the total protein metabolism

TABLE 1 (Series 2)

Experiments in the post-absorptive state

DATE (1929)	RESTING METABOLISM*			WORK DONE†	EXCESS METABOLISM OF EXERCISE AND RECOVERY					CALORIES REQUIRED TO MOVE 1 KG. 1 M.	
					½ hour period‡		1 hour period‡				
	O_2	Calories	Non-protein R.Q.		O_2	Calories	O_2	Calories	R. Q.	Based on excess metabolism of ½ hour period‡	Based on excess metabolism of 1 hour period‡
	gm. per hr.			*kgm.*	*gm.*		*gm.*				
February 25	3.78	12.92	0.94	3294	2.06	7.41	2.11	7.61	0.95	2.25	2.31
February 26	3.90	13.30	0.92	3332	2.02	7.16	2.27	8.16	0.88	2.15	2.45
February 28	4.08	13.58	0.81	3538	2.68	9.07	3.06	10.13	0.81	2.56	2.86
March 4	3.78	12.90	0.92	3459	2.40	8.37	2.43	8.46	0.86	2.42	2.45
March 6‖	4.04	13.34	0.79	3529	2.45	8.15	3.19§	10.42§	0.76	2.31	2.95
Average	3.92	13.20	0.87	3430	2.32	8.03	2.61	8.96	0.85	2.34	2.60

* Average of metabolism before exercise and after recovery.

† Running on horizontal treadmill for 10 minutes at about 1.3 to 1.4 miles per hour.

‡ Refers to the duration of the period following the beginning of exercise.

§ Somewhat restless during the second half-hour period.

‖ High fat diet on the day before this experiment.

after epinephrin and when no epinephrin had been injected, the average N excretion being about 80 mgm. per hour.

EXPERIMENTAL RESULTS. 1. *Experiments in the post-absorptive state.* In the present series, five experiments were carried out in the post-absorptive state, each being followed on the same day by an experiment after the injection of epinephrin. The post-absorptive experiments (table 1) may be compared with those of the previous series, performed many months before. The resting metabolism was somewhat higher, averaging 13.2 cals. per hour as compared with 12.7 cals. The amount of work necessary to do 1 "horizontal kilogrammeter" of work was practically the same as before,

being 2.34 cals. as compared with 2.40 cals. when the calculation was based on the first half-hour after the commencement of exercise; and 2.60 cals. as compared with 2.67 cals. when the calculation was based on the first hour after exercise.

The resting respiratory quotient was definitely higher, as the animal received the ordinary maintenance diet each day, whereas in the earlier series, as has already been stated, the post-absorptive experiments were as a rule preceded by a day on which nothing but fat was administered. It should be noted that the higher resting R.Q. (0.87) was reflected in an

TABLE 2 (Series 2)

*Experiments after epinephrin**

DATE (1929)	RESTING METABOLISM†			WORK DONE‡	EXCESS METABOLISM OF EXERCISE AND RECOVERY‖					CALORIES REQUIRED TO MOVE 1 KG. 1 M.	
					½ hour period§		1 hour period§				
	O_2	Calories	Non-protein R.Q.		O_2	Calories	O_2	Calories	R.Q.	Based on excess metabolism of ½ hour period§	Based on excess metabolism of 1 hour period§
	gm. per hr.			*kgm.*	*gm.*		*gm.*				
February 25	5.20	18.02	0.97	3491	3.84	13.76	4.56	16.77	0.98	3.94	4.80
February 26	5.22	17.74	0.87	3505	3.32	11.57	4.14	14.30	0.94	3.30	4.08
February 28	5.72	19.34	0.85	3701	3.86	13.53	5.01	17.45	1.01	3.66	4.72
March 4	5.34	18.30	0.91	3494	3.21	11.47	4.43	15.49	0.92	3.28	4.43
March 6¶	6.08	20.14	0.80	3594	4.24	14.22	5.56	18.52	0.82	3.96	5.15
Average	5.51	18.71	0.88	3557	3.69	12.91	4.74	16.51	0.93	3.63	4.64

* 1.0 mgm. (about 0.1 mgm. per kilo) injected subcutaneously 1½ hours before exercise

† See table 1.

‡ See table 1

§ See table 1.

‖ Above the resting level in the post-absorptive state.

¶ High fat diet on the day before this experiment.

equally higher R.Q. of the excess metabolism of exercise, this being now 0.85.

2. *Experiments after epinephrin.* In these five experiments, 1.0 mgm. of adrenalin chloride (corresponding to about 0.1 mgm. per kilo, as the dog weighed about 9.5 kilos) was injected subcutaneously. A preliminary period of ½ hour to permit comparatively stable conditions to become established in the apparatus was followed by two ½ hour resting periods, two ½ hour periods of exercise and recovery, and subsequent resting periods. A resumé of these experiments is to be found in table 2. It will be noted

that both when calculated on the first ½ hour after the beginning of exercise and on the first hour, there is a marked increase in the number of calories required to do 1 "horizontal kilogrammeter" of work over that which obtained when no epinephrin had been administered. A concise comparison of these two sets of experiments will be seen in table 3.

TABLE 3 (Series 2)

Comparison of R.Q. and heat production in "post-absorptive" and epinephrin experiments

	RESTING NON-PROTEIN R.Q.	R.Q. OF EXCESS METABOLISM OF EXERCISE	RESTING HEAT PRODUCTION	ENERGY CONSUMPTION PER KGM. OF WORK
			calories per hr.	*calories*
February 25:				
Basal	0.94	0.95	12.9	2.25
Epinephrin	0.97	0.98	18.0	3.94
Difference			39%	75%
February 26:				
Basal	0.92	0.88	13.3	2.15
Epinephrin	0.87	0.94	17.7	3.30
Difference			33%	54%
February 28:				
Basal	0.81	0.81	13.6	2.56
Epinephrin	0.85	1.01	18.3	3.66
Difference			42%	43%
March 4:				
Basal	0.92	0.86	12.9	2.42
Epinephrin	0.91	0.92	18.3	3.28
Difference			42%	36%
March 6:				
Basal	0.79	0.76	13.3	2.31
Epinephrin	0.80	0.82	20.1	3.96
Difference			52%	72%
Average:				
Basal	0.87	0.85	13.2	2.34
Epinephrin	0.88	0.93	18.7	3.63
Difference	0.01	0.08	42%	55%

In the first place, epinephrin injection results in a marked "calorigenic" action, the resting heat production being elevated, on an average, 42 per cent above the basal level. This is a true stimulation of the cell metabo-

lism, in that it is not dependent upon an increased restlessness of the animal. The work of previous investigators using anesthetized animals would in itself indicate that this is so, and in the experiments here reported, the dog was quiet during the resting periods whenever observed. In certain experiments that I performed, and which I am not reporting because they are not particularly germane to the present paper, epinephrin was administered in doses as high as 0.2 mgm. per kilo. A record was taken of the animal's movements by means of a sensitive recording device. The record indicated that, while restlessness when present undoubtedly contributed, as would be expected, to the elevation of metabolism induced by epinephrin, the effect was obtained even when the amount of movement was not greater than under basal conditions. However, such an observation is at best not quantitative, and the most convincing evidence that muscular work is not the cause of the "calorigenic" action of epinephrin lies in the fact that this "calorigenic" effect was completely summated with the extra metabolism of exercise, which would certainly not have been the case had the dog's restlessness been the source of the increased heat production following epinephrin.

The summation referred to was complete. On the average, it required 55 per cent more energy for the animal to do a unit of work after epinephrin than when no epinephrin had been injected. Thus the 42 per cent "waste heat," seen in the resting condition, was not at all utilized for the performance of muscular work.

A comparison of the respiratory quotients (table 3) is of some interest. It will be seen that the average basal quotient of 0.87 was raised to a negligible extent by epinephrin over a period of about 3½ hours after the injection, the average resting R.Q. after epinephrin being 0.88. This is at variance, it must be confessed, with the observations of most previous investigators, and indicates that in this particular dog, at least, there was very little more utilization of carbohydrate by the animal at rest following epinephrin than when it had not been administered.

However, in the case of the quotient of the excess metabolism of exercise and recovery, there did seem to be a little greater oxidation of carbohydrate after epinephrin than before, and than in the resting condition following epinephrin. The divergence is not marked, and is dependent largely, as will be seen, (table 3) upon an excess quotient of 1.01 obtained in a single experiment. Nevertheless, as between the excess metabolism of the pre- and post-epinephrin states, it is fairly consistent, though still sufficiently slight so that I would not lay too much stress upon it. Considering the relatively large increase ordinarily observed in the blood sugar after injection of epinephrin, the comparatively small effect upon the R.Q. is rather surprising.

In the fact that the specific dynamic action of sugar, as was shown in part

I of this paper, was clearly used in muscular exercise, whereas the excess energy exchange resulting from epinephrin administration was not, there lies additional evidence, if that were needed, that the stimulating action of of epinephrin is not due to the dynamic effect of carbohydrate plethora.

This sharp differentiation between the two above mentioned types of stimulation, the fact that in its failure of utilization as a source of free energy in exercise the calorigenic effect of epinephrin resembles the waste heat of protein metabolism, and the further fact that as shown by Rapport and Beard (1928), the specific dynamic action of protein is not to be dis-

TABLE 4 (Series 2)

Comparison of experiment in the post-absorptive state and after tyrosine

DATE (1929)	RESTING METABOLISM*			WORK DONE†	EXCESS METABOLISM OF EXERCISE AND RECOVERY§					CALORIES REQUIRED TO MOVE 1 KG. 1 M.	
					½ hour period‡		1 hour period‡				
	O_2	Calories	Non-protein R.Q.		O_2	Calories	O_2	Calories	R.Q.	Based on excess metabolism of ½ hour period†	Based on excess metabolism of 1 hour period†
	gm. per hr.			*kgm.*	*gm.*		*gm.*				
Experiment in the post-absorptive state											
April 19	3.68	12.54	0.88	3469	2.44	8.54	2.80	9.62	0.91	2.46	2.77
Experiment after tyrosine (15 gm.)											
April 19	4.43	14.95	0.85	3402	2.80	9.58	3.34	11.17	0.84	2.82	3.28

* See table 1.
† See table 1.
‡ See table 1.
§ See table 1.

tinguished from that of its constituent amino acids, led to the speculation that the calorigenic action of epinephrin might be closely related to amino acid stimulation, and particularly to that of tyrosine. Accordingly I performed an experiment with this amino acid.

3. *Experiment after tyrosine.* On April 19 a control experiment in the post-absorptive state was carried out, followed on the same day by one in which exercise was performed after the administration of 15 grams of tyrosine (Pfanstiehl) by mouth. The results are summarized in table 4. In the post-absorptive state the animal had a resting metabolism of 12.5 cals., which was increased to 15.0 cals. in the pre- and post-exercise resting periods following the ingestion of tyrosine—a specific dynamic action of 20

per cent. In the post-absorptive state the dog required 2.46 cals. (calculated on the first ½ hour after exercise) to do 1 "horizontal kilogrammeter" of work; while after tyrosine this amount of work required 2.82 cals., an increase of 15 per cent. Calculated on the 1 hour period after exercise, the calories required to do work increased from 2.77 to 3.28, or 18 per cent. In other words, as in the case of epinephrin, the excess energy following the administration of tyrosine was not used in muscular exercise and recovery. Considering the close chemical relationship of epinephrin and tyrosine, and the possibility that the former is derived from the latter in the body, this may be significant, though it would be dangerous to labor the point at present.

Discussion. The evidence presented in the two parts of this communication indicates that the specific dynamic action of carbohydrate and fat is not necessarily waste energy and that these foodstuffs are not only used, but used economically, in muscular exercise. When the animal is at rest, the stimulation of the cells to extra energy production results in exothermic reactions which appear as heat, and as such are useless for the performance of work. In muscular exercise, however, the energy of these reactions is utilized, and since the reactions involved the consumption of oxygen, it may be assumed that they are utilized in the recovery process of muscular contraction. This would appear to betray a difference between the metabolism of contracting muscles and that of the resting organism. Yet it is not safe to assume that this difference is other than quantitative, for it is at least possible that in the resting state an unknown proportion of the extra energy resulting from the stimulation of these foodstuffs may serve as a source of *free* energy, and that the extra heat of the specific dynamic action may not represent the total stimulating effect upon the cell metabolism. Be this as it may, it remains as a curious fact that for the purposes of muscular exercise, the extra energy is utilized without loss, as the practically complete absence of summation indicates. This 100 per cent efficiency is probably illusory, however, and simply means that the utilized energy is replacing energy that in its absence would be derived from other sources. It is 100 per cent substitution rather than 100 per cent efficiency, and the true efficiency with which the extra energy is utilized by the muscle in this case is the same as of those reactions which it replaces.

Even the apparent *quantitative* difference between the use of this energy by muscles and by other tissues may not represent a true difference in metabolic processes, for it may be that the specific dynamic action of carbohydrate and fat is obtained, when the animal is at rest, solely because the resulting increased energy is beyond the immediate needs of the body, and that *any* increased necessity for free energy whether from the muscles or from some other source, would abolish the specific dynamic action of these foodstuffs.

In view then of the fact that not only can both carbohydrate and fat serve as fuel for the recovery process of muscular contraction, but that the so-called "waste heat" of their metabolism may also be similarly utilized, the non-specificity of the exothermic portion of the recovery process becomes evident. But it is equally clear that this non-specificity is not absolute. Not all exothermic reactions can serve for this purpose, for not only must the specific dynamic action of protein be regarded as "waste heat" both at rest and during exercise, but also that of tyrosine, as well as the calorigenic effect of epinephrin.

SUMMARY

1. The specific dynamic action of fat, as well as that of glucose, is abolished during muscular exercise and recovery; in other words, this extra energy, which at rest appears as waste heat, is utilized as free energy in muscular work.

2. This tends to confirm the non-specificity of the oxidative recovery process of muscular contraction.

3. On the other hand both the "calorigenic" action of epinephrin and the specific dynamic action of tyrosine, like that of protein, cannot be used in muscular work. Hence the above mentioned non-specificity is limited, as certain oxidative exothermic reactions will not serve for this purpose.

4. Incidentally, the experiments confirm the conception that the calorigenic action of epinephrin is due neither to muscular work nor to the specific dynamic action of carbohydrate.

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STUDIES ON ADRENAL INSUFFICIENCY

IX. The Influence of Extracts of Adrenal Cortex (Sheep and Cattle) on the Survival Period of Adrenalectomised Dogs and Cats

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In paper V of this series (Rogoff and Stewart, 1928) the influence of dogs' adrenal extracts in lengthening the period of survival in dogs after total adrenalectomy (in two stages) was demonstrated. A not inconsiderable number of the treated animals survived far beyond anything seen in untreated controls. The period of good health seems to be correspondingly prolonged, perhaps relatively even more than the total survival period. Not seldom the animal continues to eat almost up to the end, so that it may be impossible to notice the onset of anorexia as a definite event presaging death, as is usual in the controls some days before death. At present we do not deduce a positive conclusion from a modification of the symptomatology, although we think this may be observed, unless in a considerable number of the animals the survival period is increased without question beyond the limits seen in the controls. We have also gained the impression that the pathological changes in the gastro-intestinal mucosa are modified by treatment with extracts, hemorrhagic congestion in the mucosa being less common and less extensive. This may be the case even in animals which do not outlive controls. The congestion of the pancreas is not affected.

In table 1 are assembled the results of observations on 32 dogs treated by intravenous injections of extracts of cortex (interrenalin) from abattoir material, in this case entirely from sheep. The injections were made once daily, the dose varying from 1 to 2 cc. The injections were well borne, no ill effects being seen. Six dogs lived beyond the maximum period seen in the controls ($16\frac{1}{2}$ days, $17\frac{1}{3}$ days, $17\frac{3}{4}$ days, over 19 days, $20\frac{1}{2}$ days, $22\frac{1}{2}$ days). Four dogs lived practically for the maximal period of the controls ($14\frac{5}{6}$ days, 15 days, $15\frac{1}{4}$ days, $15\frac{1}{2}$ days). The ten unusually long survivors were in a series of but 32 dogs. A much smaller proportion of animals running beyond 14 days was seen in the controls (at least 200 in number)—only 2 in table 1, paper I (Rogoff and Stewart, 1926) in 29 male dogs; none in 39 non-pregnant female dogs in table 2, paper I; and 4 in 36 dogs in

table 2, paper V (1928). Altogether, only 6 dogs in the 104 control dogs in these three tables lived over 14 days and none reached 16 days. It is clear that the treated adrenalectomised dogs present us with a new picture,

TABLE 1

Adrenalectomised male dogs treated with extracts of sheep's adrenal cortex (intravenously)

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation					Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*				
132-4	9.3	9.1	23	11½		10	+−	0	++++	
132-5	10.9	10.4	23	12½		12	+	+−	+++	
132-6	8.8	8.7	23	19	2	17½	+	+	++++	
133-6	10.8	10.6	24	10¾		9	+	+−	++	Small ulcers (pylorus and duodenum)
133-7	11.1	12.3	24	7½		5	+	+	+	Coughing at 2nd operation
133-8	8.2	8.5	24	15½		15	0	0	++++	
133-9	9.6	10.0	27	11¾		11	0	0	+++	
134-5	11.2	11.0	27	7¾		6½	++	++	+++	Small erosions (pylorus)
134-6	9.4	9.8	21	10¾		9	0	0	+++	
135-9	11.3	11.7	27	20½		19	+	+	++++	
136-0	7.5	8.2	27	7¾		5½	0	+	+++	
136-2	14.9	14.0	16	13¾		13	0	0	++	
136-3	10.8	10.8	16	10	20½	9	++	+	++++	
136-4	13.3	13.5	16	8¾		7	0	0		
137-0	11.8	12.1	36	8	22	6	+++	+	++	7 small ulcers (pylorus); 1 larger ulcer (duodenum)
137-1	11.8	11.8	36	16½		14½	++	+	+	
137-4	19.9	19.4	30	22½		21	0	+−	+	Small erosions (pylorus)
137-5	15.3	13.5	41	13	22½	11	+−	+−	+++	Very mangy
137-6	15.9	16.3	41	15	0	15	0	0	+++	Erosions (pylorus)
149-8	9.0	9.6	35	9¾		8½	+	+	++++	
149-9	11.8	12.3	35	13	15	10	+	+	+++	Many small ulcers (pylorus)
150-0	11.1	11.0	31	17¾		15	0	0	+++	
150-2	10.6	11.3	30	10¾		10	+++	+++	++	
150-3	13.2	13.2	30	8½		7½	0	0	++++	
150-4	14.8	15.2	29	12	0	9	++	++	+++	
156-2	17.7	19.4	18	10	2	5	++	+++	++++	Ulcers (pylorus and duodenum)
156-3	18.2	17.0	18	14	20	13½	+−	+−	+++	
156-5	16.0	16.9	17	15	6	15	++	++	++++	
160-1	18.3	19.9	69	8½		6	+++	+++	++++	
162-0	15.4	16.2	36	12	0	10	+++	+++	++++	Many ulcers (stomach)
162-1	16.0	15.5	36	17	8	15½	++++	++++	++++	
162-2	16.5	16.3	36	6	0	4	++++	++++	+++	

something not seen in the controls in the relatively large number of animals surviving decidedly beyond the maximum observed in the controls. Long survivals not surpassing the maximum are more common than among the

controls. If table 1 is analysed on the same basis as table 1, paper V (1928), which contains the results with intravenous injection of extracts of dogs' adrenals into dogs, six animals are found to surpass the maximum in the controls, the total number of animals being practically the same for the two kinds of material. In addition, however, there are in table 1 of the present paper four animals which lived from $14\frac{5}{6}$ to $15\frac{1}{4}$ days, whereas there are none in this group in table 1 of paper V. That table contains 7 dogs

TABLE 2

Adrenalectomised male dogs treated with extracts of sheep's adrenal cortex (subcutaneously)

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED	BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Blood	Congestion		
	kgm.	*kgm.*	*days*	*days hours*	*days*				
132-3	9.8	9.2	23	$15\frac{1}{2}$	$14\frac{1}{2}$	0	+−	+	
134-1	11.2	11.0	27	$7\frac{1}{2}$	$6\frac{1}{2}$	++	++	+++	Small erosions (pylorus)
134-6	8.9	9.1	21	10 $22\frac{1}{2}$	10	0	0	+++	
137-2	9.9	8.4	37	$10\frac{1}{2}$	8	+−	+	++	
137-3	17.5	16.1	37	$13\frac{1}{2}$	12	0	0	++	Ulcers (pylorus and duodenum)
138-6	19.3	18.9	19	7 $21\frac{1}{2}$	6	0	+	+++	
138-7	14.4	14.3	33	$9\frac{1}{2}$	7	++	+	+++	Many small ulcers (pylorus); large ulcer (duodenum)
139-0	11.1	10.1	28	$8\frac{1}{2}$	8	0	+−	++	
139-1	15.4	14.8	28	9 12	9	0	+	+	Mangy
139-3	17.2	16.4	29	7 5	4	+++	++	+++	Goiter. Spleen enormously congested
141-0	14.4	13.4	20	10 18	9	0	+	++++	4 small ulcers (pylorus)
141-4	13.4	12.7	36	23 $3\frac{1}{2}$	21	++	++	+++	Old dog. Ulcers (pylorus)
141-5	15.4	15.0	38	6 3	3	++++	++++	+++	
141-7	9.9	8.1	37	$6\frac{1}{2}$	$5\frac{1}{2}$	0	+	++	
141-9	11.4	11.7	22	$14\frac{1}{2}$	14	0	+	+++	3 ulcers (pylorus)
143-4	19.6	19.2	28	$4\frac{1}{2}$	$2\frac{1}{2}$	+++	++	++	
143-5	13.4	12.8	28	7 $1\frac{1}{2}$	$6\frac{1}{2}$	+	+	++++	Erosions (pylorus)
143-6	12.5	12.4	28	$11\frac{1}{2}$	9	+	+−	+++	Small erosions (pylorus and duodenum)
143-8	9.3	9.1	28	$11\frac{1}{2}$	8	++++	++++	+++	

surviving from 11 days to $13\frac{1}{2}$ days, against 12 dogs living from practically 11 days to 14 days in table 1 of the present paper. It is clear that there is no essential difference between the results of intravenous injection of homologous and heterologous material. Nor should any difference be expected from what is known of active substances from other endocrine glands. That no animal in the present series approached the exceptional survival period of 78 days seen in one dog of the previous series may be considered accidental. It is not known what favorable circumstances

enabled that animal to live so long. The important point is that it was ascertained that no remnant of adrenal tissue was left behind, nor did the most careful and prolonged search reveal any accessory adrenal. The results of our extensive control series leave no doubt that the long survival was due to administration of the extracts.

The period of good health is lengthened fully as much as the total survival period. Not seldom the animal continues to eat, at least some food, till within a short time before death (often 1 day or less). We do not trust much to averages of survival periods for reasons given in previous papers, but the average is about 13 days for the treated animals in table 1.

TABLE 3

Adrenalectomised male dogs treated with extracts of sheep's adrenal cortex (by mouth in coated capsules)

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED	BEGAN TO REFUSE FOOD	ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Blood	Congestion		
	kgm.	*kgm.*	*days*	*days hours*	*days*				
146–1	10.0	9.9	47	8¼	7½	+++	+++	+++	Several small ulcers (pylorus)
146–2	10.0	11.1	48	10¼	6	+++	+++	+++	
146–3	14.8	15.0	35	11 ½	9	++++	++++	++++	Erosions and ulcer (pylorus)
146–4	14.0	13.6	46	8 20	7	+++	+++	++	
146–6	9.1	10.2	46	6¼	5	++++	++++	++	
149–0	11.6	11.1	27	11½	10½	+++	+++	++++	Ulcers (pylorus and duodenum)
149–1	10.0	9.8	27	9 5	7	++++	++++	+++	
149–4	13.6	13.2	23	10 19½	9½	++++	++++	+++	
149–5	12.7	13.3	24	9 21	7	++++	++++	++	
149–6	11.7	11.4	22	16 3	14½	+++	+++	++++	Capsules and dropper; ulcers (duodenum)
150–1	12.4	11.6	31	11 7½	10½	++	++	++++	Ulcers (pylorus)
156–4	17.3	17.6	16	13¾	12	+++	+++	++++	Ulcers (stomach)

In table 2 are assembled the results of subcutaneous administration of cortical extracts (sheep). The table includes 19 dogs. The extracts were similar to those injected intravenously. Only one dog (141-4), obviously an old animal, survived beyond the maximum of the controls, living indeed over 23 days, or a week more than any control dog. Two other dogs lived beyond 14 days ($14\frac{3}{4}$ and $15\frac{2}{3}$). Save for the dog which survived 23 days the result would be considered negative. The average survival period for the 19 dogs is 10.4 days. The average of the control dogs in table 2, paper V, is 9.6 days. The result may perhaps be considered as slightly positive, but subcutaneous administration of our extracts in dogs appears to be much inferior to intravenous administration. It is quite possible that,

judged by this very severe test of an increase above the maximum survival period (extension of survival beyond the maximum seen in the controls), subcutaneous injection may give a negative result in the great majority of the cases with an exceptional positive result. We have formed the impression that old animals usually bear the loss of the adrenals better, i.e., survive longer than young animals. This is not ascertained, it is, as said, an impression.

In table 3 are included a small series of 12 dogs treated orally with the same extracts as used for the animals in tables 1 and 2 except that the proteins were not precipitated and the dose was greater. In nearly all cases the extracts were given in coated capsules, expected to pass through the stomach without being destroyed. Sometimes the dogs were with difficulty induced to swallow the capsules. Some dogs would discover them in the food and carefully eliminate them. On the whole, however, there is no doubt that they all took the chief part, at least, of the doses given them. Occasionally recourse was had to pouring the extract into the back of the pharynx with a medicine dropper.

The results in table 3 are negative. Only one of the animals lived as long as 16 days. Two lived $13\frac{2}{3}$ days. Three lived 11 to $11\frac{1}{2}$ days; two about $10\frac{3}{4}$ days. All the others, save one which lived nearly 7 days, survived $8\frac{3}{4}$ to $9\frac{7}{8}$ days. The average is 10.7 days, but means less even than usual on account of the small number of animals.

In the course of the last five years, we have tested extracts obtained by different methods from the adrenal cortex with the view of determining whether they could substitute for the adrenals and thus prolong the life of adrenalectomised dogs and cats. During four or five years preceding this period we made numerous tests with cortical extracts upon adrenalectomised rabbits and guinea pigs. Various solvents were employed. Among the methods employed was precipitation by various salts and subsequent extraction of the precipitates, fractional precipitation, dialysis, etc. We did not obtain favorable results with the salt precipitates in the relatively small number of animals on which they were tried at that time. In view of the statement of Hartman and his associates (1928) that the life of adrenalectomised cats can be prolonged by subcutaneous administration of extracts obtained by precipitation with sodium chloride, we have made experiments to test this statement. Everything was done precisely in the way described by these observers, including control of the pH (in the case of the acetic acid extract electrometrically, and of the final product colorimetrically and electrometrically). The preparation employed by us was that designated by them as VIIIa. This represents the extract from which they obtained the best results. We shall refer to this extract as a salt precipitation extract. Fresh material was prepared twice a week. The dosage was the same as administered by them.

Table 4 contains results on 32 adrenalectomised cats treated subcutaneously with extracts of cortex of cattle prepared in this way. One of the cats (166-2) was in advanced pregnancy at the time of the first adrenal

TABLE 4

Adrenalectomised cats treated subcutaneously with extracts made by salt precipitation

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED	BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right		
	kgm.	*kgm.*	*days*	*days hours*	*days*	*gram*	*gram*		
163-3	3.4	3.4	15	7 5	6			+	Small gastric ulcers
163-4	3.2	3.2	15	7¼	6			+	
163-5	1.5	1.7	29	8½	7½			++	
163-6	3.2	3.2	15	7½	7			+	
163-7	4.1	4.0	15	9½	8			+++	
163-8	3.6	3.6	28	17½	15½		0.90	+++	Adrenals calcified
163-9	3.2	3.1	28	8¼	7½			++	Ulcers (pylorus)
164-0	2.2	2.4	28	6½	6			+	
164-1	2.4	2.5	28	23½	20			+++	
164-2	2.5	2.8	28	15½	13			++	Ulcers (stomach)
164-8	2.9	2.9	42	8 5½	4	0.23	0.30		
164-9	3.9	3.6	42	7½	6	0.35	0.24	++	
165-0	3.7	3.8	42	31½	29	0.22	0.23	0	Old cat
165-3	3.0	3.3	44	7½	5	0.16	0.20	++	Ulcer (pylorus)
165-4	3.1	3.3	44	8 0	3	0.15		+++	
165-9	2.4	2.3	34	8½	6½	0.11	0.10	+++	Early pregnancy at first operation
166-0	2.8	2.7	34	13½	12	0.23	0.25	++	
166-1	3.1	2.9	34	6½	4	0.15	0.17	++	Ulcer (pylorus)
166-2	2.5	2.1	60	23 0	22	0.17	0.16	+++	Advanced pregnancy at first operation
167-0	2.9	2.8	15	8½	8	0.17	0.16	+++	
167-2	3.7	3.8	15	7 4	5½	0.24	0.22	+−	
167-3	3.3	2.9	15	6½	5½	0.16	0.16	++	Small erosion (stomach)
167-4	2.8	3.0	15	13½	12	0.12	0.12	+++	Ulcers (pylorus)
167-6	1.9	2.1	15	12 1½	11	0.10	0.12	++	
167-7	2.9	2.7	15	11½	8	0.23	0.27	+−	
167-8	2.9	3.0	15	17½	15½	0.12	0.17	++	
167-9	2.9	3.0	19	4½	4	0.26	0.33	+++	Small erosions (stomach)
168-0	3.5	3.5	19	7 23½	7	0.30	0.37	+++	
168-5	3.2	3.1	28	12 0	11½	0.14		++++	Ulcers (pylorus)
168-8	2.5	2.3	28	6½	5½	0.12	0.12	+	
168-9	2.3	2.1	28	14½	13½	0.16	0.14	++	Ulcer (pylorus)
169-0	2.9	2.7	28	7½	6½	0.15	0.16	+++	Ulcers (pylorus and duodenum)

All the cats were male except 164-0, 164-1, 164-2, 165-9 and 166-2.
In 163-8, 165-9 to 166-2 and 167-6 to 168-0 inclusive the left adrenal was removed first; in the others the right.

operation and 18 days thereafter gave birth to 4 kittens. She lived 23 days after removal of the second adrenal, the gland having been excised 42 days after delivery. At autopsy the uterus was found incompletely involuted

and corpora lutei prominent. It is not known whether the pregnancy (and heat) exercised any effect upon the duration of survival, but the animal did not reach the maximum survival period seen in our control cats (Rogoff and Stewart, 1929). We have considered it fair to place this cat in table 4 in spite of the possible influence of the pregnancy so as to give the method being tested the benefit of any doubt.

Cat 165-9 was in early pregnancy at the time of the first operation. Three days after the operation she miscarried and gave birth to 5 embryos $2\frac{1}{2}$ to 3 cm. long. The second adrenal was removed 34 days after the first operation. The animal lived $8\frac{2}{3}$ days.

If the table is compared with our control tables for cats (Rogoff and Stewart, 1929) it is seen that there is no essential difference. The maximum survival period in table 4 is $31\frac{1}{2}$ days. This was in an obviously old cat. Although there may be some reason to suspect that very old cats

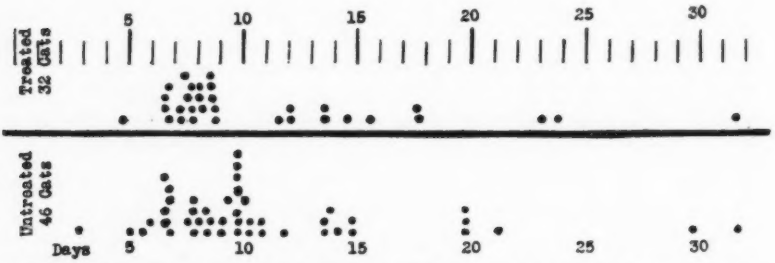

Fig. 1. The upper part of the chart represents the survival periods of our 32 cats (table 4) treated by subcutaneous administration of cortical extract prepared by precipitation with sodium chloride. The lower part of the chart shows the survival periods of our 46 control, untreated cats.

and dogs bear loss of the adrenals better than younger animals this is not certain, and it would have been unjustifiable to exclude the animal from the table on this ground.

The maximum survival period ($31\frac{1}{2}$ days) is exactly the same as in the control tables, from which two old castrated males were excluded. One of these lived slightly over 35 days, the other only $7\frac{1}{2}$ days. In table 4 one cat reached $23\frac{5}{6}$ days. Among the controls one cat lived $29\frac{3}{4}$ days, and one-eighth of all the animals survived about 20 days or longer. In table 4 three, i.e., one-tenth of the cats lived 20 days or longer. Among the controls more than one-fifth lived 2 weeks or longer, and among the treated cats less than one-sixth survived 2 weeks or longer. Between one-third and one-fourth of the treated cats lived 13 days or longer, and one-fourth of the controls did likewise. About three-eighths of the treated animals survived 9 days or longer, as compared with more than four-

sevenths of the controls. The average for the 32 treated cats was 11.1 days; for the 26 male controls 11 days; and for the 20 non-pregnant female controls $10\frac{3}{4}$ days. It is obvious that in every particular the results on the cats in table 4 treated with extracts by salt precipitation are the same as those in the untreated control series. The influence of the treatment was therefore completely negative.

The practical identity of the results in our treated cats and in our control series of untreated, adrenalectomised cats is shown very clearly in figure 1. The upper part of the graph is plotted from table 4 of this paper and the lower part from tables 1 and 2 of paper VIII. The numbers represent

TABLE 5

Adrenalectomised male dogs treated subcutaneously with extracts made by salt precipitation

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED	BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation				Left	Right	Blood	Congestion		
	kgm.	*kgm.*	*days*	*days hours*	*days*	*gm.*	*gm.*				
162–8	7.3	7.8	114	10½	8	0.47		++	++	+++	Ulcers (pylorus and duodenum)
164–6	8.2	9.0	67	4½	4½	0.62		+	++	++++	Ulcers (pylorus)
165–6	10.4	10.4	72	4½	3	0.66	0.70	+	++	+–	Ulcers (stomach)
166–5	9.2	9.4	60	3½	3½	0.53	0.54	+++	++	++	
166–7	8.6	8.7	34	6½	4	0.60	0.44	0	+	++++	Ulcer (duodenum)
166–8	11.3	10.6	34	11 3½	9½	0.55	0.49	+++	++++	++	Ulcers (stomach)
168–2	7.2	6.7	22	8½	7	0.41	0.43	0	+	+++	Erosions (pylorus)
168–3	11.4	11.2	22	10½	9½	0.65	0.61	0	0	+++	
169–1	9.8	10.1	21	12 4	9	0.79	0.78	++	++	++++	
169–2	8.7	8.7	21	9½	1	0.57	0.50	+	+	++	Ulcers (pylorus and duodenum)

In 165–6, 168–2 and 168–3 the left adrenal was removed first; in the others the right.

the days the animals lived after the second adrenalectomy. In both series the great bulk of the points lie between 5 and 10 days; a smaller number, between 10 and 15 days; one or two, less than 5 days; one or two, about 30 to 32 days. It must be remembered that the lower (control) series contains about 50 per cent more animals than the upper (treated) series.

The routine diet for cats (both control and treated) consisted of milk in the afternoon of the day of operation, the adrenalectomy being done in the forenoon. Milk was usually given also the day after the operation. Thereafter, fish or boiled meat on alternate days with raw meat. The boiled meat was in the form of a stew with potatoes, etc. Milk was given when the other food was refused.

Table 5 contains the results of subcutaneous injection, into dogs, of extract made by salt precipitation. None of the dogs lived as long as the maximum period of the controls. Four lived 10 to 12 days. Three lived less than 5 days. The average was about 8¼ days for the 10 animals comprised in the table. The result is therefore entirely negative.

In table 6 are comprised 12 dogs treated intravenously with the same material. One of the dogs lived 25 days, 5 hours, and three lived about 2

TABLE 6

Adrenalectomised male dogs treated intravenously with extracts made by salt precipitation

RECORD NUMBER	WEIGHT		TIME BETWEEN OPERATIONS	SURVIVED		BEGAN TO REFUSE FOOD	ADRENAL WEIGHTS		ALIMENTARY CANAL		PANCREAS CONGESTION	REMARKS
	First operation	Second operation					Left	Right	Blood	Congestion		
	kgm.	*kgm.*	*days*	*days*	*hours*	*days*	*gm.*	*gm.*				
162-3	14.2	13.1	91	13¾		12½	0.87		+	++	++++	Ulcers (pylorus)
162-4	6.0	6.8	81	8	3½	7½	0.42		+++	+++	+++	Wt. 6.5 kgm. near end
162-6	11.6	11.4	50	11	1	10			0	++	++++	
163-1	19.2	17.5	49	11	21	9½			+++	+++	++++	Large ulcers (duodenum)
163-2	11.7	10.2	84	5	12	4		0.70	++	++	++	Large ulcers (stomach and duodenum). Wt. 9.7 kgm. at end
164-5	10.5	9.5	40	13¾		13½			++	++	++++	
165-8	13.3	12.6	41	25	5	24½	0.88	1.19	++	++	++	
166-6	11.3	11.4	34	7¾		6	0.62	0.52	+++	+++	+++	Ulcers (pylorus)
166-9	9.6	8.9	34	9¾		6	0.62	0.55			++++	Blood in stools. Wt 8.5 kgm. at end
169-4	7.5	8.0	21	11	5	9½	0.43	0.45	+	++	++++	Stomach much congested
169-7	11.3	11.2	20	14	1	11½	0.66		+	+	++++	Stomach blood clots and small erosions
169-8	8.0	9.2	20	11		10	0.72	0.77	+	+++	++++	Small ulcers (stomach)

In 163-1, 163-2, 165-8 and 169-4 the left adrenal was removed first; in the others the right.

weeks. Of the 12 dogs in the table, eight, or two-thirds, survived 11 days or longer. The average for the 12 animals was 12 days. The results are decidedly inferior to those obtained with extracts prepared according to our method and exhibited in table 1. But although the number of dogs is too small to be quite satisfactory, we might interpret the results as slightly positive. It does not seem unlikely that extracts which probably contain only a relatively small proportion of interrenalin should exhibit some power of prolonging life when injected intravenously, even if the results of subcutaneous injection were negative.

Accessories. It is self evident that great care must be taken in all work involving adrenalectomy to determine whether accessories are present, which can be seen and removed at operation or are only discovered at autopsy. In dogs, as we have already pointed out, accessories are quite rare but this is not so in cats. We have determined the frequency with which accessories were found in a large number of cats. In one group of 55 cats there were 5 in which an accessory was found and removed at the time of the operation. In another group of 66 cats there were two in which accessories were discovered and removed at operation. In the 121 cats there were seven with accessories, or about 6 per cent. This anatomical fact can be applied to test the thoroughness of the search for accessories in large series of cats. For instance, in a recent paper (Hartman, et al., 1928) describing experiments on a very large number of cats it is not stated that accessories were found in any of them although "in animals which survived a week or more careful post-mortem search was made for the presence of accessory cortical bodies." According to our statistics, it may be assumed that a certain number of the animals in this series possessed accessories. If this is so, the few cats which are stated to have survived beyond the longest survival period in our control, untreated cats, may have done so in virtue of the presence of accessories and not in virtue of the potency of the extracts with which they were treated.

Ulcers. We mentioned some years ago (Rogoff and Stewart, 1926) the frequency of gastric and duodenal ulcers in adrenalectomised dogs. In adrenalectomised cats ulcers are very common. In 46 control adrenalectomised cats there were ulcers in the stomach or duodenum or both in 14 animals. In 32 cats treated subcutaneously with extract prepared by salt precipitation there were 11 with ulcers. In 7 pregnant adrenalectomised cats there were 4 with ulcers. Altogether in 89 cats there were 29 with ulcers. That is, about one-third of the animals had ulcers. In a previous paper (Rogoff and Stewart, 1929) it was stated incidentally that ulcers in the stomach are more common in cats than in dogs after adrenalectomy. The proportion in dogs was given in an earlier paper (Rogoff and Stewart, 1926) as at least one-sixth of the cases. Larger experience and perhaps more careful search have shown that the proportion is higher than this in dogs and that there is much the same incidence in the two kinds of animals. For instance, in 118 adrenalectomised dogs, 48 had ulcers, about two-fifths of all the cases. The ulcers in the majority of cases extend through the mucosa. They are often quite large. Sometimes they are superficial, and these are usually small and multiple. The larger ulcers are also often multiple. Sometimes the large ulcers appear to have been formed by the confluence of smaller ones, giving the appearance of an irregular outline. The larger ulcers more frequently extend into or through the muscular coat, and not seldom only the peritoneum remains

unperforated. Occasionally they have perforated the peritoneum. In cats ulcers seldom extend as deeply into the muscular coat. In 32 unoperated cats, which were used for other experiments or died of causes other than loss of the adrenals, we found an ulcer in the stomach in only one animal. The great frequency of ulcers after adrenalectomy points to the same conclusion as the other pathological changes in the alimentary canal described by us in earlier papers, that the loss of the adrenals is apt to be associated with serious derangement in the gastro-intestinal tract.

SUMMARY

In paper V of this series proof was given that extracts of adrenal cortex from dogs (interrenalin) can prolong the period of survival of dogs after adrenalectomy beyond the maximum seen in control untreated animals. It was stated further that similar results were obtained in dogs by intravenous injection of cortical extracts from sheep's adrenals. The data on the influence of these extracts are given in the present paper. Subcutaneous administration of the extracts did not exert as favorable an influence as intravenous injection.

Extracts prepared from the adrenal cortex of cattle in the manner recently described by Hartman and his co-workers and administered subcutaneously, as in their experiments, were found to have no influence on the survival period of adrenalectomised cats, as compared with our untreated controls. These extracts, injected intravenously into dogs, were not nearly so effective as our own extracts. If they produced any definite effect at all, it was slight. Subcutaneous administration in dogs had no favorable influence upon the survival period.

Accessories were found in about 6 per cent of 121 cats operated on for removal of the adrenals.

Ulcers in the stomach or duodenum, of adrenalectomised dogs and cats, are very common. Of 118 adrenalectomised dogs, 48, or two-fifths of the cases, had one or more ulcers. Of 89 adrenalectomised cats, 29 had ulcers (about one-third of the cases). Of 32 normal cats, only one had an ulcer (in the stomach.)

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BLOOD PRESSURE AND PLASMA PROTEIN DETERMINATIONS IN THE SAME FROG[1]

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If filtration of water and crystalloid substances in the blood occurs in the glomerulus, as is postulated in Cushny's *Modern Theory of Renal Function*, it must be shown, in the case of the frog, that there is sufficient blood pressure to overcome the osmotic pressure of the colloids.

Varying results have been reported in the literature for the percentage of protein in frog's plasma; for example: Halliburton (1886) 2.54 per cent; Scott and Bieter (1922) 0.6–0.8 per cent; White (1924) 2.4 per cent (equal to 10–12 cm. of H_2O osmotic pressure), and Landis (1927), again equal to an osmotic pressure of 11.5 cm. of H_2O. The same variation exists in blood pressure studies in the frog as can be seen from the following figures: Volkmann (1850) 22 and 29 mm. Hg, aortic pressure; Hill and McQueen (1921) 5–10 mm. Hg pressure definitely slowed the glomerular capillary flow; and Hayman (1927) 15.4–45 mm. Hg — average 27.3 ± 4.7 mm. Hg for systolic aortic pressure, 11–40.4 mm. Hg — average 23.1 ± 4.4 mm. Hg for afferent glomerular artery pressure, and 2.9–38.2 mm. Hg —average 14.8 ± 5 mm. Hg for glomerular capillary pressure. If now one takes the lowest glomerular capillary pressure and the highest plasma protein percentage, one finds that there is not enough blood pressure to counterbalance the osmotic pressure of the proteins. Because of this, it was thought advisable to investigate the problem further and obtain readings for blood pressure and plasma protein percentages in the same series of frogs.

In the first place, it was found necessary to devise some means of determining blood pressure in the intact frog. A rubber cuff was constructed, to which was attached a mercury manometer, a Woulfe bottle to serve as

[1] A summary of the findings was reported to the Minnesota Branch of the Society for Experimental Biology and Medicine (see Proc. Exper. Biol. and Med., 1928, xxv, 832).

The experiments herein reported form part of the thesis, "Contributions to the physiology of the kidney," submitted in May 1929 by Raymond N. Bieter, in partial fulfilment of the requirements for the degree, Doctor of Philosophy.

a buffer, a valve from the radiator of a household hot water heating system to serve as an outlet valve, and an atomizer bulb for inflation. The rubber cuff with a cloth wrapper was wrapped around the frog's leg. The principle for the apparatus was borrowed from the ordinary mercury manometer used for clinical determinations of blood pressure.

The blood pressure cuffs were constructed out of small rubber balloons, varying in size from $\frac{1}{2}$ inch wide and 3 inches long for small frogs (Rana pipiens), to 1 inch wide and 5 inches long for large frogs (Rana catesbiana). To one end a small rubber tube was cemented at right angles. A cuff has also been constructed for this work by the Taylor Instrument Corporation, measuring 1 inch wide by 3 inches long, together with a cloth binder similar to those used clinically. The cuff was wrapped firmly around the thigh or the leg. To help keep it in place, flexion of the leg on the thigh, or of the foot on the leg, almost to right angles, prevented the cuff from sliding downwards. (Fig. 1 shows a photograph of the apparatus.)

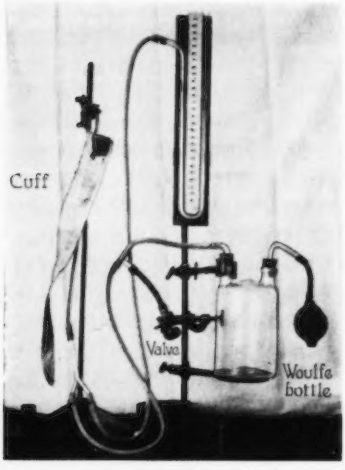


Fig. 1

To hold the frog during the procedure of determining the blood pressure, a small box with a slit on one side to allow the right leg to protrude, was constructed. The size of the box curbed the frog's movements, but did not keep it in a cramped position. Moist paper padding helped to restrain its movements.

Arterial blood flow was determined by placing the web of the extremity to which the cuff was applied, under the low power of an ordinary microscope, using either direct or indirect illumination. The largest arteriole in a web was generallly used for the observations. Throughout the work the same part of the foot was used, namely, the median central web, looking at the foot from the dorsal surface. When studying the blood pressure in large frogs, the toes were held spread apart with the left hand upon the stage of the microscope, leaving the right hand free to manipulate the bulb and release valve. For small frogs, a small frame was made on a piece of galvanized iron and the foot was held with the toes spread apart with small clamps specially made for the purpose over an opening to allow light to pass through the web.

When an arteriole of the web was brought into focus, a pulsating stream could be seen. The pressure in the cuff, which is recorded by the mercury

manometer, was now raised until pulsations could no longer be observed. A continuous flow was to be noted for a time which in most cases gradually disappeared, probably due to the contractility of the arteries, generally leaving stasis in a vessel of reduced diameter. In a large number of cases the continuous flow slowed gradually and then changed to a reverse flow, but when equilibrium was reached, stasis again occurred. The pressure in the system was now gradually reduced and when two or three synchronous pulsations were first noted, the mercury manometer was read for systolic pressure. As the pressure was then allowed to drop further it was noticed that between the peaks of systolic pressure, the blood stream slowed in velocity, and that as the pressure dropped still further, this decrease in velocity between systoles became an actual arrest of flow which again in many cases became a reverse flow. When this marked retardation, arrest or reversed flow first began to disappear, and was converted into a suddenly increasd onward flow toward the veins, the mercury manometer was read for the diastolic pressure. At this point the red blood cells showed an increased onward movement.

When frogs were prepared as above and blood pressure readings were taken, it was found that in most cases the pressures were relatively high; for example: 50–70 mm. Hg systolic, and 35–48 mm. Hg diastolic. As the frogs became settled in their surroundings, however, the readings decreased to 20/16–40/28 mm. Hg. If readings were taken daily for a period up to six days, it was found that the pressures did not decrease further. These low uniform readings over a period of days, within a range of 2 to 3 mm. Hg, have been taken to be the correct readings for a given frog. It has also been observed that struggling of the frog was able to cause a rise of pressure equal to 10–15 mm. Hg, but that during the following half hour, as a rule, it receded to its original low or basal level; example: basal pressure 30 mm. systolic/20 mm. diastolic; struggle 38/26; struggle 46/35; fifteen minutes later 38/24; thirty minutes later 32/20. Excessive stimulation such as noise has also caused a similar increase in blood pressure. Occasionally it has also been noticed that the blood pressure rose rather suddenly (5–10 mm. Hg) and then remained stationary for 10 to 15 minutes or more. During many of these variations, when the frog box was opened suddenly, the frog was found filled up with air, so it appears that in this manner the frog is able to cause an increase of its blood pressure. The blood pressure readings reported in this paper have all been taken on frogs kept in a stationary water bath at temperatures of from 20 to 25°C. The heart rates have been between 30 to 50 per minute. Some readings have been obtained on frogs taken from a cold running water tank, with heart rates around 15 to 22 per minute, in which blood pressures have run from 35/16 to 50/21 mm. Hg, showing a greater pulse pressure at this heart rate. Because of all of these variations, blood pressure readings

were only considered as normal when readings for several days on frogs in the same surroundings were found to be within several millimeters of mercury of each other.

A number of determinations of blood pressure have been made on both large and small frogs before, during and after ether anesthesia. In table 1 can be seen the effect of placing a frog under a bell jar in an atmosphere

TABLE 1

Effect of ether anesthesia on blood pressure in the frog

FROG NUMBER	BLOOD PRESSURE											
	Before			During anesthesia			Fifteen minutes later			Thirty minutes later		
	mm. Hg			*mm. Hg*			*mm. Hg*			*mm. Hg*		
1	35/29	35/28	35/28	24/10	24/10		29/22	30/23	29/25	30/25	32/25	32/25
2	42/38	39/34	39/34	18/15	20/17	20/17	29/25	30/25		36/32	36/32	38/34
3	44/34	44/36		32/24	34/26	32/24	38/30	38/30	38/30	48/38	46/38	

TABLE 2

Calibration of cuff blood pressure readings with Huerthle and Harvard membrane apparatus

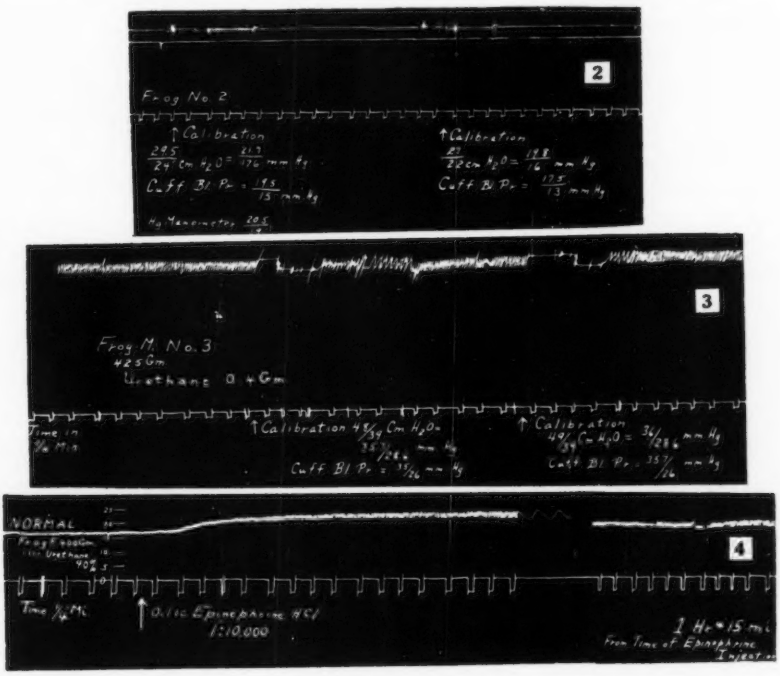
FROG NUMBER	WEIGHT	NORMAL BLOOD PRESSURE	ANESTHETIC	BLOOD PRESSURE THIRTY MINUTES LATER		CUFF BLOOD PRESSURE	HUERTHLE AND HARVARD READINGS cm. H_2O = mm. Hg
	gm.	*mm.* Hg				*mm.* Hg	
1	460	40/28 38/28 38/28	Na Luminal 125 mgm.	34/26 36/26 36/28	Frog prepared for blood pressure tracing record	30/22	44/32 = 32.3/23.5
						33/24	48/36 = 35.3/26.4
						30/23	43/32 = 31.6/23.5
2	480	38/28 38/26 37/26	Na Luminal 150 mgm.	32/22 34/22 30/20		19.5/15	29.5/24 = 21.7/17.6
						17.5/13	27/22 = 19.8/16
						32/22	53/41 = 39/30
3	425	42/26 42/26 43/26	Urethane 400 mgm.	33/26 32/26 33/26		35/26	48/39 = 35.7/28.6
						35.7/26	49/39 = 36/28.6
						33/26	47/38.5 = 34.5/28.3
4	470	38/24 38/26	Urethane 400 mgm.	32/26 34/28		32/24	46/36 = 33.8/26.4
						36/26	51/39 = 37.5/28.6
5	500	44/32 46/34 46/34	Urethane 400 mgm.	35/26		34/27	49/40 = 36/29.3
						36/26	49/40 = 36/29.3

of ether until struggling disappeared, and upon removal, the frog remained perfectly flaccid.

The effects of sodium luminal and urethane are shown in table 2.

To prove the correctness of the blood pressure readings in the preceding pages, tracings were obtained of frog's blood pressure, using Huerthle and Harvard membrane manometers, and also a mercury manometer,

attached to cannulae in the coeliac artery of five large frogs. These readings were taken immediately after the blood pressure tracing was made, and in some cases during the recording of the manometers. The frogs were anesthetized with sodium luminal or urethane. Coagulation of blood was inhibited by a subcutaneous injection of heparin (5 mgm.) made before the operative procedure, and also by placing $\frac{1}{2}$–1 mgm. of heparin in the saline used to fill the cannulae before attachment of the manometers. The surgical procedure used to prepare the frogs for the



Figs. 2, 3 and 4

experiment was the same as that described by Bieter and Hirschfelder (1924).

Table 2 gives the results of the calibrations. In the table, reading from left to right, are shown: frog no. and weight, normal cuff blood pressure in millimeters of mercury, amount of anesthetic used, lowered blood pressures following anesthetic injection, and lastly, the calibrations of the Huerthle and Harvard manometer readings compared with the cuff blood pressures, taken as close to the time of the tracing as possible. The figures for the

blood pressure obtained in this manner through the coeliac artery were from 0 to 2.3 mm. Hg higher than those obtained by the cuff method for systolic pressure, and from 0.5 to 3.3 mm. Hg higher for the diastolic pressure. One exception to this is calibration no. 3 of frog 2 which was obtained following the intravenous injection of 0.2 cc. of epinephrine HCl 1:10,000. In this case the cuff blood pressure was 32/22 and the membrane manometer gave a calibrated reading of 39/30 mm. Hg. Concerning the main group of readings and calibrations, it is believed that the figures for systolic and diastolic blood pressures obtained by the cuff method are within the limit of error.

TABLE 3

Collection of blood samples and approximate volumes

FROG NUMBER	DURATION OF BLEEDING OF SAMPLES				APPROXIMATE VOLUME OF SAMPLES		
	(1)		(2)	(3)	(1)	(2)	(3)
	Rana pipiens						
	minutes	*seconds*	*minutes*	*minutes*	*cc.*	*cc.*	*cc.*
1	30				3		
2	[illegible]				2.5		
3		50	30		2	2.5	
4	1		25		1.2	3	
5	3				2		
6	1		15		3	2.7	
	Rana catesbiana						
7	1	10	35		3	3.5	
8	1	30	25		3.2	3	
9	1		30		1.5	4	
10	2	45	30		3.5	4	
11	1		3	12	4.5	4	5
12	1	30	15		4	5	

Figure 2 shows the coeliac blood pressure tracing from frog 2 obtained with a Huerthle manometer. Figure 3 shows the same type of tracing from frog 3 obtained with a Harvard manometer. Both tracings also show calibrations (1) and (2) for each frog together with the cuff blood pressure readings taken immediately after the membrane manometer records were obtained. In addition, the first calibration of frog 2 shows the blood pressure obtained with a mercury manometer immediately after. Figure 4 is included to illustrate the type of tracing obtained with a mercury manometer. This tracing also shows the effect of an intravenous injection of 0.2 cc. epinephrine hydrochloride 1:10,000. As can be seen from the figure, the effect of this injection had not completely worn off at the end of $1\frac{1}{4}$ hours.

Following the determinations of normal blood pressure in a series of small frogs (Rana pipiens) and in a series of large frogs (Rana catesbiana) these frogs were bled for plasma protein determinations by the method of precipitation with trichloracetic acid solution. One-half to one hour before the bleeding, the frogs were injected subcutaneously with heparin to prevent coagulation of the drawn blood. The small frogs were given 1 to 2 mgm. and the large frogs 4 to 6 mgm. Preparatory to bleeding, the frogs were slightly etherized, but not to the point of complete relaxation of muscles. The blood was collected from the small frogs directly from the heart. The animal was pinned to a board, the heart exposed, and then inverted, the tip of the ventricle cut off, and the blood then collected in small glass tubes. In the case of the large frogs, cannulae were inserted into the coeliac artery, the animals turned sideways to an angle of 45° and the blood collected from the cannulae.

Table 3 shows the length of time during which the various samples of blood were collected and their approximate volumes. The first samples in those frogs where two or more were collected, were obtained over a period of time varying from ½ to 3 minutes, and in most of the cases probably represents the closest approximation to normal frog's blood. The second sample in the case where three were collected was obtained over a period of three minutes. The final samples ((2) in most of the frogs and (3) in frog no. 11) were collected over periods of time varying from 12 to 35 minutes.

The blood samples were immediately centrifuged after collection, the corpuscle volume estimated roughly, and the clear, yellowish plasma pipetted off. Then accurately measured plasma samples, as large as could be obtained from each blood, were placed in weighed glass tubes. These plasma samples varied from ½ to 2 cc. To the plasma in the weighed tube was now added trichloracetic acid (10 per cent) to about two or three times the plasma volume. The tubes were agitated and stirred with a capillary glass rod and after standing for at least thirty minutes, they were centrifuged. The clear liquid was poured off, the precipitates washed again with trichloracetic acid, centrifuged, and then washed twice with 70 per cent alcohol.

Following the last washing and centrifuging, the tubes containing the precipitate were placed in a drying oven at 102°C. until a constant weight was obtained. This was usually reached in two or three days.

The normal blood pressure readings, and the percentage of plasma protein or trichloracetic acid precipitate and corpuscle volume, for the two series of frogs (Rana pipiens and Rana catesbiana), are shown in tables 4 and 5, respectively.

Scott (1916) showed that in mammals, after hemorrhage, the fluid entering the blood is practically pure salt solution. This same fact is to be

noted here, at least as far as protein capable of being precipitated by trichloracetic acid is concerned. In the case of the frogs of both species, the protein percentage was lower in the second and third blood specimens as was also the case with the corpuscle volumes. The most marked drop in plasma protein can be seen in the plasmas of frog 11 where the first plasma protein amounted to 4.3 per cent and the third plasma protein, or tri-

TABLE 4

Plasma proteins and blood pressure in small frogs

FROG NUMBER	BLOOD PRESSURE				PLASMA PROTEIN (TRICHLORACETIC ACID PRECIPITATE)		CORPUSCLE		
					(1)	(2)	(1)	(2)	
	mm. Hg				*per cent*	*per cent*	*volume per cent*	*volume per cent*	
1	32/24	32/24	32/24	30/23	0.535				Small frogs Rana pipiens. Weight 30–50 grams
2	33/26	34/22	33/24	33/24	2.12				
3	32/24	32/24	32/22	32/22	3	1.9	30	17	
4	36/18	36/20	35/18	36/18	3.64	3.3	30	18	
5	22/17	22/16	21/17	21/16	1.9		25		
6	32/22	30/22	28/20	28/20	2.88	2.37	30	20	

TABLE 5

Plasma proteins and blood pressure in large frogs

FROG NUMBER	BLOOD PRESSURE				PLASMA PROTEIN (TRICHLORACETIC ACID PRECIPITATE)			CORPUSCLE			
					(1)	(2)	(3)	(1)	(2)	(3)	
	mm. Hg				*per cent*	*per cent*	*per cent*	*volume per cent*	*volume per cent*	*volume per cent*	
7	30/20	32/20	32/24	30/20	2.7	1.99		25	17		Large frogs Rana catesbiana. Weight 325–525 grams
8	30/22	30/20	30/20	30/20	4.48	1.56		33	25		
9	32/20	30/18	32/20	34/20	4.0	1.47		23	15		
10	30/21	31/21	30/20	28/20	3.6	2.3		30	20		
11	36/24	36/23	34/22	34/24	4.3	4.1	0.4	27	25	15	
12	36/24	34/20	34/22	34/24	3.9	3.8		30	25		

chloracetic acid precipitate, 0.4 per cent. Referring again to tables 3, 4, and 5, it can be seen that, in general, the longer the duration of any bleeding time and the less the mixture of the first or more normal blood, with the blood flowing into the tube after the first three or five minutes, the greater is the decrease in plasma protein.

The lowest plasma protein, or trichloracetic acid precipitate determina-

tion in Rana pipiens was 1.9 per cent, and the highest, 3.64 per cent, the average of five determinations being 2.71 per cent. The lowest blood pressure in the same species of frog was 21 mm. Hg systolic and 16 mm. Hg diastolic; and in the same units, the highest blood pressure was 33/26, the average from six frogs being 30.5/21.1 mm. Hg.

The lowest plasma protein determination in Rana catesbiana was 2.7 per cent and the highest 4.48 per cent, the average of six determinations being 3.83 per cent. The lowest blood pressure in the same species of frog was 28/20 and the highest 36/24 mm. Hg, and the average from six frogs was 32/21.2 mm. Hg.

These results show that the frogs of the species Rana pipiens have a slightly lower blood pressure and a slightly lower plasma protein, or trichloracetic acid precipitate percentage, than the frogs of Rana catesbiana. Another important point is that in all of the frogs studied, the blood pressures are slightly lower in the frogs showing the lowest plasma protein percentage.

The highest figure given by Krogh (1922) for the osmotic pressure of frog's plasma is 40 mm. H_2O for 1 per cent of protein. White (1924) and Landis (1927) give practically the same figures. In terms of mercury, 40 mm. H_2O are equal to 2.9 mm. Multiplying this figure by the highest per cent of plasma protein, 4.48, in the large frogs, gives 13.99 mm. Hg as its osmotic pressure. It can be seen that this is below the average diastolic pressure in the large frogs which is 21.2 mm. Hg, and well below the diastolic pressure in the animal mentioned above, which is 22 to 20 mm. Hg.

Hayman (1927) found that the glomerular capillary pressure was equal to 54 per cent of the systolic aortic pressure. Fifty-four per cent of the average systolic pressure found in the large frogs, namely, 32.8 mm. Hg is equal to 17.2 mm. Hg. This, then, may be considered as the average glomerular capillary pressure. The average plasma protein for the large frogs was found to be 3.83 per cent, which gives an osmotic pressure of 11.1 mm. Hg. This figure is again below the average figure of 17.2 mm. Hg for the diastolic glomerular capillary pressure, which is probably the lowest blood pressure obtained in the glomerular capillaries. The same relation is to be found in the small frogs. Fifty-four per cent of the average systolic pressure, namely, 30.5, is equal to 16.47 mm. Hg for the average diastolic glomerular capillary pressure. The average plasma proteins in these same frogs, which is 2.71 per cent, gives an osmotic pressure of 7.85 mm. Hg.

CONCLUSIONS

1. A new method for obtaining values of blood pressure in the intact frog has been described.

2. These findings obtained by this method are thought to be within the

limits of error since they have been confirmed by blood pressure tracings produced with calibrated Huerthle and Harvard membrane manometers.

3. Normal blood pressures in a series of frogs shows an average blood pressure for Rana pipiens equal to 30.5/21.1 mm. Hg, and for Rana catesbiana, 32/21.2 mm. Hg.

4. Blood pressure values in frogs show rather marked variations as a result of struggling and excitement, as is the case in mammals and man.

5. Plasma protein percentages obtained by precipitation with trichloracetic acid show for Rana pipiens an average of 2.71, and for Rana catesbiana an average of 3.83.

6. Plasma proteins and blood pressures in frogs tend to show some variations, but the variations in a given frog seem to affect both values in the same direction.

7. Fluid drawn into the blood in frogs after hemorrhage appears to be protein-free, just as is the case in mammals.

8. The findings of Hayman (1927) for general systolic blood pressures in frogs have been confirmed. The averages given here are higher than those of Hayman, however. This, in all probability, is due to the fact that the frogs used by Hayman were anesthetized, or pithed, and prepared surgically for the observations. Such procedures tend to reduce the general blood pressure.

9. There is sufficient diastolic blood pressure in the frog to over-balance the osmotic pressure of the proteins. Using Hayman's figure to determine diastolic glomerular capillary pressure, there is again sufficient blood pressure to over-balance the osmotic pressure of the proteins.

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AN ATTEMPT TO AUTO-TRANSPLANT THE ADRENAL[1]

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Auto-transplants of practically every tissue in the body have been attempted with much success. Auto-transplantation of the adrenal, however, has not met with such success. Since Ivy and Farrell (1925-1926) were so successful in transplanting a portion of the stomach and pancreas subcutaneously by using a pedicle-graft method, it was thought that this method might be used with success on the adrenal.

The most recent attempt to transplant adrenal tissue was made by Zwemer (1927) who transplanted cortical tissue into the rectus abdominis muscle, or beneath the tunica vaginalis of the testis, or into the peritoneum. He found that the grafts were uniformly unsuccessful, and he believed the length of life of his cats was prolonged due to the resorption of the hormone from the degenerating graft, which is questioned by the recent observations of Rogoff and Stewart (1929), on the survival time of adrenalectomized cats. Elman and Rothman (1924) ligated the lumbo-adrenal veins and destroyed by blunt dissection the small blood vessels of one adrenal. They found that this adrenal atrophied except for some medullary tissue and the other adrenal hypertrophied. The removal of the hypertrophied adrenal resulted in death in spite of the presence of some normally staining medulla in the atrophied gland. Attempts at transplantation of the adrenal by Boinet (1895), Hultgren and Anderson (1899) and Strehl and Weiss (1901) yielded negative results. Stilling (1905) found that transplants made into the testicle of rabbits would sometimes persist for one and a half and two years, but their physiological functioning was not proven. Poll (1899) and H. and A. Christiani (1902) made subcutaneous, muscular and peritoneal transplants on rats. The latter observers had one animal survive for eleven months, but did not know that some rats survive bilateral adrenalectomy. Schmieden (1903), working on rabbits, found that adrenal transplants into the kidney might survive anatomically as long as one year. Busch, Leonard, and Wright (1908) made direct auto- and homo-transplants of one-third of an adrenal

[1] A preliminary report by Oldberg and Ivy: This Journal, 1927, lxxxi, 488.

TABLE 1

Summary of results of adrenal auto-transplantation into omentum

TRANSPLANTATION TO OMENTUM	LENGTH OF TIME OF ADRENAL ON THE PEDICLE BEFORE REMOVAL OF RIGHT ADRENAL	TIME OF SURVIVAL ON PEDICLE	TIME OF SURVIVAL ON AUTO-TRANSPLANT	CAUSE OF DEATH	ANATOMICAL RESULTS—GROSS AND MICROSCOPIC
	days	*days*	*days*		
Dog 1—Female†	20	15	1	Adrenal insufficiency	Normal grossly; glomerulosa normal; medulla degenerated; cortex—much degeneration
Dog 2	7	10	*	Distemper	Normal grossly; medulla degenerated except in 1 area. Most of cortex and all glomerulosa normal
Dog 3	9	2	*	Adrenal insufficiency	Normal grossly; glomerulosa normal; cortex and medulla degenerated
Dog 4	23	9	*	Adrenal insufficiency	Normal grossly; parts of medulla normal; cortex and glomerulosa normal
Dog 5	20	8	*	Adrenal insufficiency	Normal grossly and microscopically
Dog 6	32	10	*	Adrenal insufficiency	Nodule 5 mm. in diameter. Normal cortex and medulla
Dog 7	21	1	*	Adrenal insufficiency, toxic absorption	Adrenal necrotic and surrounded by mass of omentum
Dog 9	46	18	*	Adrenal insufficiency	Cortex normal except for small areas of anemic infarcts. Medulla normal
Dog 10	26	69	6	Peritonitis, ruptured duodenal ulcer—2 ulcers	Round cell infiltration beneath capsule; cortex normal; medulla normal
Dog 11—Female†	26	69	4	Adrenal insufficiency	Recent ischemic necrosis
Dog 12	72	44	10	Adrenal insufficiency	Grossly normal; no histology
Dog 16	103	5	*	Adrenal insufficiency	Ischemic necrosis

TABLE 1—*Concluded*

TRANSPLANTATION TO OMENTUM	LENGTH OF TIME OF ADRENAL ON THE PEDICLE BEFORE REMOVAL OF RIGHT ADRENAL	TIME OF SURVIVAL ON PEDICLE	TIME OF SURVIVAL ON AUTO-TRANSPLANT	CAUSE OF DEATH	ANATOMICAL RESULTS—GROSS AND MICROSCOPIC
	days	*days*	*days*		
Dog 17	70	1	*	Adrenal insufficiency	No transplant found
Dog 18	69	2	*	Adrenal insufficiency	Ischemic necrosis
Dog 20	60	14 months	3	Adrenal insufficiency	Vessels to transplant definitely enlarged, 3 veins measuring about 1.5 mm. in diameter; normal grossly

* Dog did not survive long enough to cut pedicle.

† Non-pregnant, no oestrus.

into the kidney through an incision in the capsule, in rabbits. They had two rabbits which survived the removal of the other adrenal, but which died, soon after the removal of the kidney containing the adrenal transplant, one living two days, the other forty-three hours. Histologically, adrenal transplanted tissue was in the kidney. Their work, in the light of the more recent work on the survival time of adrenalectomized rabbits, is not conclusive. Their rabbits should have lived longer. The most conclusive observations from a physiological viewpoint are those of Haberer and Stoerk (1918). They pseudo-transplanted one adrenal of dogs and rabbits on a pedicle into the kidney and found that after removal of the other adrenal the animal would live long periods of time, one dog living as long as nine years. The pedicle was not cut. On removing the kidney containing the adrenal transplant, the animals died.

METHODS AND RESULTS. Twenty-five male dogs were used in this study. Transplants were made into the omentum of 19 dogs, 4 of which died of distemper before removal of the right adrenal and are not included in table 1. Transplants were made into the spleen of three dogs (dogs 22, 23, and 25). In two dogs (14 and 15) transplants were made to the jugular vein. In one dog the lumbo-adrenal vein was tied on each side of the adrenal bilaterally.

The results in table 1 show that of the fifteen dogs only four (dogs 10, 11, 12 and 20) lived long enough to demonstrate that their "pedicle-pseudo-transplant" was functioning. It might be argued that dogs 1 and 9 who

survived on the pedicle 15 and 18 days were getting some benefit from the pedicle-pseudo-transplant, but since dogs occasionally (Rogoff and Stewart) survive adrenalectomy for 15 days we doubt that the pedicle was performing much, if any, function.

Of five dogs that survived long enough on the pedicle-pseudo-transplant to warrant its section, one lived 1 day, one 3 days, one 4 days, one 6 days, and one 10 days following section of the pedicle. These survival times are not long enough to show that the auto-transplant was functioning. Dog 10 probably would have lived longer had he not died of peritonitis

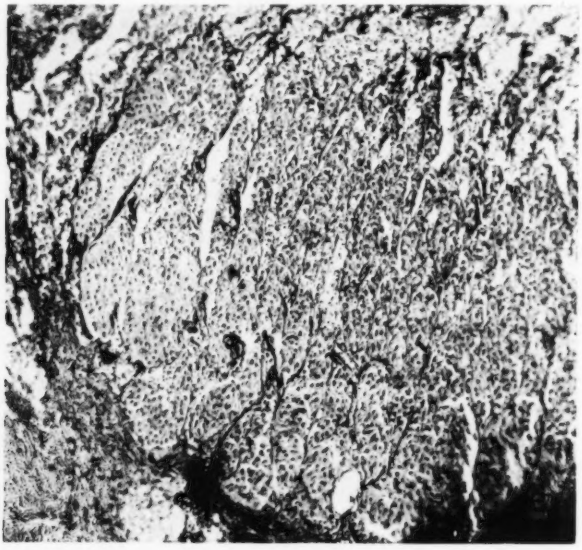

Fig. 1. Showing normal zona glomerulosa of the pseudo-transplant nine days after removal of the other adrenal and thirty-one days on the pedicle. Dog 4.

caused by the rupture of a duodenal ulcer. This animal had two ulcers in his duodenum of the "kissing" or contact type.

Histological and gross studies of the adrenal-pseudo-transplant showed that of the fifteen dogs all but five had sufficient anatomical adrenal tissue to live. Dog 3 did not; he lived only two days. Dog 7 did not; he lived only one day and the adrenal had been entirely absorbed leaving a cavity filled with serosanguineous fluid which was surrounded by a mass of omentum. Dog 17 did not; he lived only one day, the adrenal being entirely absorbed. Dog 18 did not; he lived only two days and the adrenal was intact grossly, but it histologically presented the appearance of an

anemic infarct. The adrenal of dog 16 which lived 5 days, presented the same appearance as that of dog 18. Dog 11 lived so long on the pseudo-transplant that the ischemic necrosis evident in the pseudo-transplant must have been due to sectioning the pedicle, which procedure produced an infarct or ischemic necrosis.

In three dogs (22, 23, and 25) the adrenal was placed within the spleen, the pedicle being retained as usual. In dogs 22 and 23 the adrenal was entirely absorbed. In dog 25 a portion of the adrenal (3 × 4 mm.) had

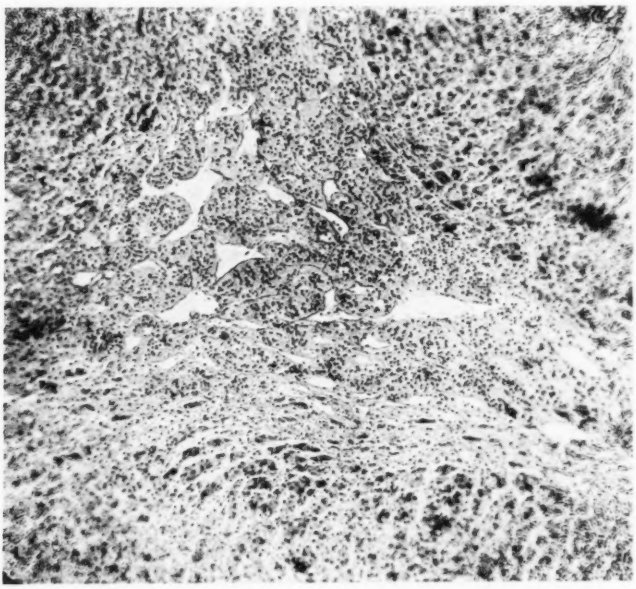

Fig. 2. Showing normal medulla of the pseudo-transplant eight days after removal of the other adrenal and twenty-eight days on the pedicle. Dog 5. The surrounding cortex is normal but out of focus.

been surrounded by omentum and was preserved, but the remainder was absorbed. This animal survived 14 days on the pedicle-pseudo-transplant. It is to be doubted that the pseudo-transplant benefited this animal.

In two dogs (14 and 15) a lateral anastomosis between the lumbo-adrenal vein and the external jugular vein was made. This was done by ligating the lumbo-adrenal vein on each side of the adrenal. Then the adrenal was removed. The external jugular was prepared and incised longitudinally as was the lumbo-adrenal vein. In one case the adrenal after incising the vein was passed through a series of beakers containing

Ringer's solution (38°C.) in an attempt to wash out the epinephrine. A lateral anastomosis was then performed by the usual blood-vessel technique using fine needles and silk thread impregnated with vaseline. Technically the operation was a success. However, a local dry gangrene resulted in both cases as if epinephrine had been injected locally. Examination of the area revealed that the external jugular vein was collapsed for some distance above and below the anastomosis, but no clot was found. Such an operation was performed to ascertain if the venous pulse would be

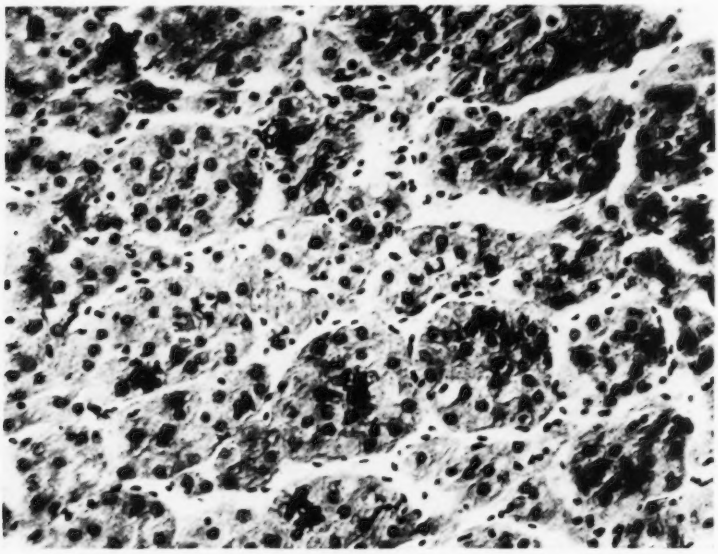

Fig. 3. Shows normal cortex of the auto-transplant sixty-nine days after removal of the other adrenal and ninety-five days on the pedicle and six days after removal of the other adrenal. Dog 10.

sufficient to furnish the adrenal with blood sufficient to preserve it anatomically. The results of these two experiments show that it will not.

The dog in which we ligated the lumbo-adrenal vein on each side of the adrenal bilaterally has lived for more than two years.

The pancreas was definitely congested in all dogs that survived more than two days. Dogs that survived more than five days showed mental disturbances and gastro-intestinal changes as described by Rogoff and Stewart.

Discussion. The results reveal several interesting phenomena which necessitate discussion, the most significant being the phenomenon that a

relatively large portion of the adrenal was preserved anatomically, but the animals died. According to Wislocki and Crowe (1924), and Biedl (1913), only a small amount of adrenal cortex is necessary for life,—about one-sixth or one-eighth. In eight of the dogs that died, we would estimate that at least one-sixth of the adrenal cortex was anatomically intact, although the medulla was degenerated in all but two. It is a well known fact that the adrenal along with the thyroid receives more blood per weight per unit of time than any other tissue in the body. This copious blood flow should be significant, and we believe that it is. If so, the fact that sufficient anatomical adrenal cortex was present in our dogs, shows that they had sufficient blood supply to preserve the anatomical structure of the cortex, but an insufficient blood supply to make possible normal psysiological function.

According to Rogoff and Stewart (1926, 1927, 1928) two-thirds of adrenalectomized dogs survive from 4 to 9 days, one-tenth from 10 to 12 days, and two out of 74 lived 15 days, several out of a series of 74 dying within 2 days. They also found that pregnancy and heat prolong the life of adrenalectomized dogs. From our experience in this series and in other work in which we have had occasion to remove the adrenals in two stages, we believe that the longest time that an adrenalectomized male dog will live is 15 days, all of our work having been done on males. Since four of our dogs on the pedicle-pseudo-transplant lived 44, 69, 69 days, and 14 months, we believe this proves that such a transplant functioned and that it received sufficient blood supply to function.

Histological examination did not yield much evidence as to the development of a secondary circulation from the omentum. Instead, in these dogs the blood vessels in the pedicle increased in size and in the dog that lived 14 months there were three veins that measured approximately 1.5 mm. in size. On sectioning the pedicle the dogs died showing that the vascularization of the adrenal from the omentum was insufficient in spite of the relatively great length of time that was allowed for the development of such vascularization. This is in sharp contrast to the results of Ivy and Farrell (1925, 1926) who observed that the transplanted pouch of the stomach and tail of the pancreas became secondarily vascularized in three weeks sufficiently not only to preserve the anatomy but the secretory activity of their transplants. The adrenal for some reason apparently "resists" secondary vascularization.

The fact that the adrenal does not or is very slow to develop a secondary blood supply is the sole factor, we believe, that prevented our experiments from being successful. The same precautions were used that were necessary for successful transplanting of the pouch of the stomach and tail of the pancreas. The pedicle graft method, we believe, is the best that can be used. The operations were performed quickly and were not at-

tended with shock. Sectioning the pedicle was done through a small incision and was the easiest of the operations performed. In other words, we believe that we gave the adrenal transplant every opportunity to function and to become secondarily vascularized, but it failed to do so adequately for the maintenance of life. It is possible that the adrenal might become more vascularized in the kidney or retroperitoneal tissues than in the spleen and omentum, but because of the marked tendency of the omentum to become fixed to and to vascularize tissue, we seriously doubt that more vascularization would occur in any other tissue. We also believe that the adrenal cannot be auto-transplanted so that its functions are maintained. However, further experiments on the kidney are indicated, in view of the work of Busch, Leonard and Wright (1908), and of Haberer and Stoerk (1918).

Our observation that dogs will survive a long time on an adrenal pedicle-pseudo-transplant into the omentum confirms the findings of Haberer and Stoerk (1918) on pedicle-pseudo-transplantation into the kidney.

SUMMARY

1. Attempts to auto-transplant the adrenal into the omentum and spleen were unsuccessful.

2. The adrenal on a pedicle consisting of the anastomosing blood vessels of the kidney capsule functioned adequately to maintain life for relatively long periods of time in four of fifteen dogs. Sectioning of the pedicle resulted in death from adrenal insufficiency in three dogs; in the fourth, the animal died on the sixth day due to a ruptured duodenal ulcer.

3. It is pointed out that the rich blood supply that the adrenal receives normally is physiologically significant.

4. The pseudo-transplanted adrenal on a pedicle does not readily develop a secondary, or a new blood supply, which is in contrast to such tissues as the skin, stomach, pancreas, intestine, etc.

I desire to express my thanks to Dr. A. C. Ivy who took part in the operative procedure.

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THE EFFECT OF HIGH FREQUENCY SOUND WAVES ON HEART MUSCLE AND OTHER IRRITABLE TISSUES

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In earlier studies on the effect of high frequency sound waves on cells and tissues Harvey and Loomis (1928) observed that frog's sciatic nerve and gastrocnemius muscle were not stimulated when touched to the side or ends of glass tubes through which intense waves of about 400 kilocycle frequency were passing. Also, stimulation of muscle, nerve, pigment cells and luminous tissue could not be obtained when touching a quartz crystal made to vibrate in air by a low power electric oscillator (Harvey, Harvey and Loomis, 1928), and in gene l stimulation did not seem possible.

A frog's heart immersed in R r's soluti n through which sound waves were passing showed an irregu y in beat but the temperature rose so rapidly that interpretation wa ubtful. The effect of high frequency sound waves on the heart has been thoroughly investigated. There is a very marked stimulating e t of these waves on a beating heart, shown by increased rate of beat, by the regular beat of a piece of previously quiescent ventricle. The effect seems to be specific for heart muscle or at least very marked in heart muscle It is not quite true, however, that nerve is always unstimulated for I have recently observed subminimal twitching of the gastrocnemius muscle when the sciatic nerve, dipping in Ringer's solution, is exposed to supersound waves passing through the solution, and smooth muscle also shows some evidence of contractions when immersed in Ringer's transmitting the waves. The effect is small and requires intense sound waves and cannot be obtained merely by touching the tissue to glass tubes or capillary rods carrying the waves.

The apparatus used was the high power one employed by Wood and Loomis (1927) and a complete diagram and general effects of the waves are described in the above paper. Suffice it to say here that a 2 kilowatt oscillator produces an electric field (50,000 volts maximum) of high frequency across a quartz crystal plate immersed in oil. The frequency can be varied by a condenser and tuned to the natural vibration frequency of the crystal which in all these experiments was 340 kilocycles per second. The vibration of the crystal is transmitted to the oil and to glass tubes

immersed in the oil. In most of the experiments the heart was in Ringer's solution[1] in a large Pyrex test tube about 6 cm wide × 26 cm. long. For beating hearts the auricles were tied with silk thread to an L-shaped glass rod support and to one arm of a heart lever arranged to write on the drum of a kymograph; for heart muscle the quiescent ventricle was tied in the same way. The test tube also contained Pt electrodes for electrical stimulation, a tube for bubbling pure oxygen, a coil of glass tubing through which ice water could be passed and a thermometer. Since the electrical field and the sound waves heat the oil and Ringer's solution, it is essential that cooling be maintained and in the experiments the temperature was not allowed to rise more than 2°C. in the region 18° to 20°C. None of the effects are due to temperature changes. A rheostat controlled the intensity of the sound waves by changing the voltage across the crystal but quantitative values for intensity cannot be given, since they depend on many uncontrollable factors.

Two species of turtle, the painted (Chrysemys picta) and the slider (Pseudyms rubriventris), and the bull-frog were used and eight hearts in all investigated. They showed essentially similar phenomena. If the sound waves are passed through beating auricles, there is an immediate increase in rate with decrease in amplitude of the beat with an occasional pause similar to the compensatory pause after electrical stimulation. Both increase in rate and decrease in amplitude occur most often immediately the waves are sent in and are most marked with the more intense waves, the rate often changing from three to four times its previous value. After exposure the normal rhythm is usually immediately attained but sometimes the beat is slower and sometimes faster. A record of the auricular contractions of the turtle regularly shows tone changes and there is frequently an increase in tone during and after exposure to supersound waves, but this change is not always constant. Figures 1 and 3 show the general effect on beating auricles; figure 3 the increased tone that results from supersonic stimulation. There is no peculiarity of the individual contraction curves resulting from supersonic stimulation observable on records taken with a rapidly moving drum.

If the quiescent apical half of the ventricle is subjected to the high frequency sound waves, spontaneous contractions begin which may be quite regular like a normal heart. They are most rapid the greater the intensity of the sound waves and stop when the sound waves stop. With low intensity supersonics the beat is rather irregular but with higher intensity the rhythm may be nearly as regular as the natural rhythm. Rhythms as high as thirty-four per minute have been observed, about equivalent to that of a normal whole heart. Figure 5 shows two rhythms obtained, the

[1] The composition of the Ringer's solution was: Na, 0.65 per cent; K, 0.014 per cent; Ca, 0.014 per cent; HCO_3, 0.02 per cent.

figure under the rhythm representing the intensity of the supersonics, eleven being high and twenty being low intensity. Figure six shows the effect of repeated electrical (induced shocks) stimulation of the turtle ventricle as compared with the effect of sound waves. It will be noted that the rhythm is the same as that from a certain rate of electrical stimulation.

In an experiment with the bull-frog ventricle which showed alternate periods of quiescence and spontaneous contraction, it was observed that sound waves sent through during a period of spontaneous contraction caused an increased rate which might stop or might continue for some time when the sound waves were turned off; and a rhythmic contraction if sent in while the ventricle was quiescent, that might stop or might beat for some time after the sound waves had ceased. This effect is no doubt connected with the condition of the heart at the time the waves are started and stopped.

Fig. 1. May 11, 1929. No. 2. Turtles' auricles beating spontaneously, showing effect of very weak, *31*, and stronger, *20*, supersonic stimulation between vertical lines on time record, which gives nine second intervals. Note no marked change in tone after exposure.

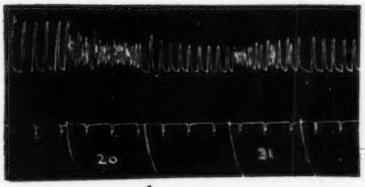
Fig. 2. May 27, 1929. No. 1. Neck muscle of small turtle showing effects of weak, *25*, and stronger, *20*, supersonic stimulation. Make and break induced shocks were sent in as marked by a jog in the signal magnet, to test irritability of tissue. Note irregular submaximal twitchings. Time five second intervals. Temperature in °C.

Fig. 3. May 12, 1929. No. 3. Large turtle's auricles beating spontaneously, showing the effect of weak, *20*, and strong, *12*, supersonic stimulation, between vertical lines on time record which gives three and seven second intervals. Temperature in °C. Note marked persistent increase in tone and occasional pauses after extra beats. Also with weak, *20*, supersonics the increased contraction rate appears only at beginning.

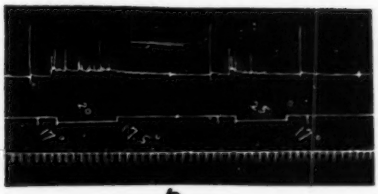
Fig. 4. May 12, 1929. No. 6. Small turtle auricles beating spontaneously in Ringer's solution in brass tube with thin brass diaphragm on bottom. High frequency electrical field of the indicated kilocycles, *KC*, between vertical lines on time record which gives three and seven second intervals. Temperature 22°C. Note that auricles beat faster only when the frequency is tuned to the natural resonance frequency, *340 KC*, of the quartz crystal plate and not when the frequency is slower, *306 KC*, or faster, *362 KC*. Electrical field intensity fairly strong, *10*, in all cases. Note rhythmic tone changes in auricles.

Fig. 5. May 27, 1929. No. 2. Quiescent ventricle of small turtle, showing effect of weak, *20*, and stronger, *11*, supersonic stimulation. Time in five second intervals. Temperature 19°C. Note more rapid rhythm with more intense, *11*, supersonics.

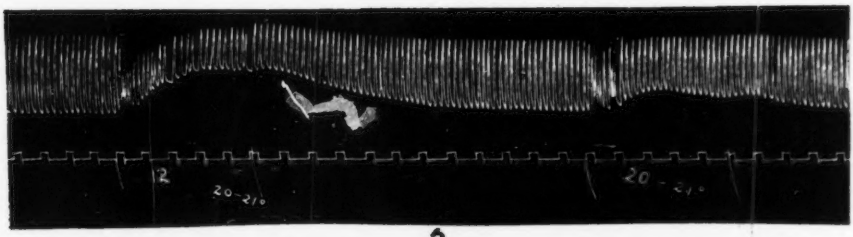
Fig. 6. May 27, 1929. No. 2. Ventricle of small turtle giving an occasional spontaneous contraction, showing effect of four rates of electrical stimulation, compared with supersonic stimulation of moderate strength, *12*. Temperature 19° to 19.5°. Time in five second intervals. From left to right, three spontaneous contractions, electrical stimulation with induction coil at rates of 153, 66, 39 per minute and with interruptor in circuit, supersonic stimulation, and a spontaneous contraction. Note similarity of supersonic stimulation to electrical stimulation at a rate of thirty-nine per minute.


20
31

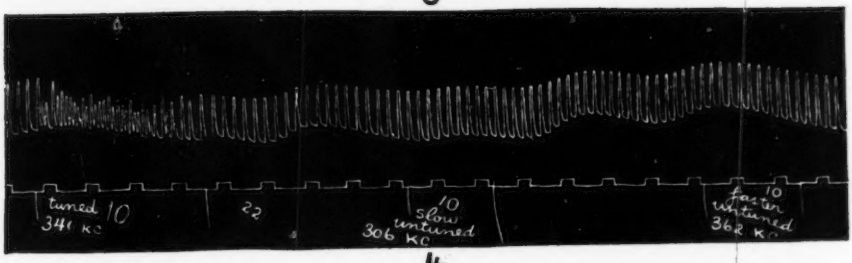
1



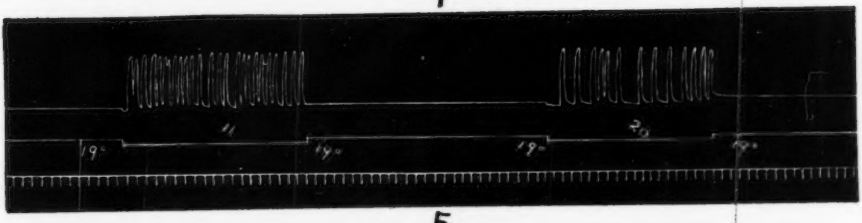
2


12
20-21°
20-21°

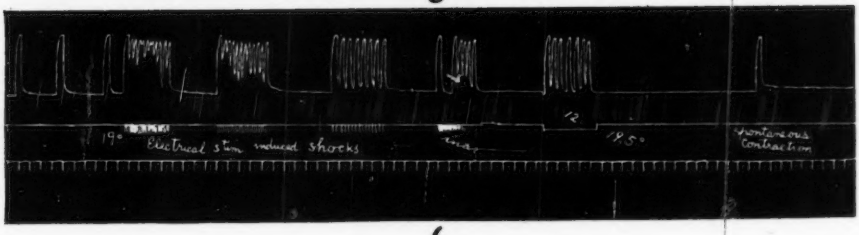
3


tuned 10
341 KC
22
10
slow
untuned
306 KC
10
faster
untuned
362 KC

4


11
19°
19°
19°
19°

5


19°
Electrical stim induced shocks
12
19.5°
spontaneous contraction

6

Since heart muscle is rather sensitive to stretching, which causes contraction, and the sound waves cause considerable movement and agitation in the Ringer's solution, it was thought that the agitation itself might be responsible for the stimulating action. This is not the case, however, for rapid bubbling of oxygen or hydrogen through the Ringer, which causes far more agitation than the sound waves, does not cause contraction of the ventricle. Neither does joggling of the stand and tube containing the ventricle cause contraction.

The next possibility was an effect of the electrical field, which might cause stimulation although the frequency is so high one should not expect it. That the electrical field is not the stimulating agent can be shown in two ways. The heart can be placed in a brass tube containing a thin brass foil membrane soldered on the bottom which is touched to the oil. Sound waves can move and pass through the brass foil readily although they do not pass up the thick brass sides and produce transverse vibrations.

If the brass tube is connected to ground there will be no field within and yet the heart in such a tube behaves like one in glass. Here we have the sound waves but no electrical field. On the other hand, if the frequency is changed the crystal will not vibrate readily as it is not in resonance. Here we have the electrical field but no sound waves and figure 4 shows that under these conditions there is no increase in rate or any effect on beating auricles. The detuned frequencies were 306 and 362 kilocycles as compared with 340 kilocycles for crystal resonance. This experiment was also carried out in the grounded brass tube so that there can be no doubt that the electrical field plays no part in the stimulation.

Another question arises as to the type of sound waves which cause the effect. If small particles (of clay or red blood corpuscles) are placed in a capillary tube sealed at one end which dips in the oil over the vibrating crystal, the particles collect in nodes of standing waves, whose distance corresponds to the half wave length of the sound in water, and is independent of the diameter of the capillary. In a wide tube the particles cannot collect in standing waves because of the vigorous currents. On the other hand oil placed on the outside of a glass capillary or rod shows nodes of standing waves whose distance is greater the wider the rod, and whose half wave length does not correspond to the velocity of sound in glass. They are nodes of transverse vibrations which in a glass test tube must be transmitted from the glass walls into the Ringer's solution resulting in a complicated wave system. These transverse waves are so weak as to be negligible in a brass tube[2] but longitudinal waves can pass up through a thin brass diaphragm soldered to the end. Transverse waves are also absent in a thin rubber bag[2] but longitudinal waves passing up through

[2] Private communication from Dr. William Richards.

the oil transverse the rubber and pass into Ringer's solution. Since the heart is characteristically affected in both the brass diaphragm tube and the rubber bag we are quite certain the longitudinal waves can produce the effect. On the other hand if heart muscle touches the glass of the glass tube the supersonic effect is most marked, due no doubt to the transverse vibrations also.

It seems rather strange that a piece of isolated ventricle attached to a writing lever and fixed to a support by a thread and suspended in air cannot be made to contract if touched to the rim of a test tube or a glass capillary carrying intense sound waves, but such is the case. If immersed in Ringer's solution the contractions appear when relatively weak sound waves are passing through. The difference must be connected with area of surface picking up the waves or with intensity. Frog nerve and muscle are also not stimulated when merely touched to vibrating glass but there is irregular subminimal stimulation when a sciatic nerve alone or a gastrocnemius muscle containing nerves is actually immersed in Ringer's solution. Striated muscle from the foot of a turtle and especially a muscle of the neck also show in the graphic records (fig. 2) subminimal irregular twitchings, which soon disappear. There was an indication of increased tone with intense supersonics in the neck muscle of the turtle together with the marked twitchings.

In one experiment a grass frog was curarized, the right leg having first been ligatured to prevent circulation of the curare in that leg. The left curarized muscle showed no twitchings when subjected to sound waves while the right uncurarized muscle shows a few subminimal twitchings. In all cases the tissue was responsive to electrical stimulation so that I conclude the weak stimulating effect of the sound waves was on the nerve, rather than muscle tissue.

Smooth muscle (stomach of frog and intestine of turtle) shows some evidence of stimulation or increased tone from the sound waves, when immersed in Ringer but the pieces studied were not very responsive to electrical stimulation.

Heart muscle is beyond doubt easily stimulated by these waves and a stopped heart could undoubtedly be started again by such treatment. It should be pointed out, however, that the possibility of passing these waves through the thoracic wall of an animal would be practically nil since they are absorbed by membranes and at phase boundaries and could not possibly pass the tissue between pericardium and exterior.

Since high frequency sound waves are like any other type of sound waves they must consist of very rapid condensations and rarefactions of the medium, the Ringer's solution, i.e., of alternate increase and decrease of pressure locally. The displacement of particles as such waves pass through will increase as the intensity increases, the energy carried varying as the

square of the amplitude. The energy also varies as the square of the frequency. This explains part of the rapid heating of the medium; the rest is due to the electrical field. Estimation of the pressure changes in a fluid carrying intense supersound waves gives values of the order of +20 to −20 atmospheres.[2]

Edwards and Cattell (1928) have observed an eight per cent increase in rate of heart beat and ninety-two per cent increase in amplitude of contraction when the whole heart is subjected to pressure changes of the order of 60 atmospheres. They have frequently observed also a few isolated beats of a temporary slow rhythm in the quiescent ventricle when subjected to increase in pressure, which is probably dependent on the level of irritability of the tissue.[3] If near the proper level for rhythmic contraction, pressure, which increases irritability, will start the rhythm. Cattell and Edwards (1928) find increase in amplitude of skeletal muscle also as a result of pressure.

Ebbecke (1924) recorded contraction of skeletal muscle when high pressure is applied and Cattell and Edwards have observed the same thing,[3] so that it is not impossible that the effect of supersonics on the heart and on muscle is connected with increase in irritability due to increased pressure. Against this view, however, is the fact that amplitude of heart contraction is not increased but diminished, if anything, by supersound waves.

SUMMARY

High frequency (340 kilocycles) sound waves of high intensity will cause rhythmic contraction of quiescent ventricular muscle of frog or turtle immersed in Ringer's solution and a more rapid natural rhythm of the auricles accompanied by lessened amplitude of contraction and often by increased tone. Touching ventricle or skeletal muscle or nerve to glass carrying intense supersound waves does not stimulate but nerves and skeletal muscle of frog or turtle containing nerves show a few subminimal twitchings of short duration when immersed in Ringer's solution. Smooth muscle of the alimentary tract exhibits some contraction from supersonic stimulation but the effect is negligible compared with that of heart muscle. Curarized gastrocnemius is not stimulated. The effect may be due to increased pressure changes accompanying the sound waves since it is known that increased pressure will stimulate.

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THE EFFECT OF EXPERIMENTAL HYPERTHYROIDISM ON THE MOVEMENTS OF THE EMPTY STOMACH

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From the Physiological Laboratories of the University of Chicago

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A fact often referred to in the discussion of hyperthyroidism is that both patients and laboratory animals appear to suffer from an increased or abnormal desire for food. A raised basal metabolic rate of from 40 to 75 per cent is one of the attributes of the Graves syndrome. As Foster (1) puts it, the increased production of heat in the body must be compensated for by increased food ingestion. The patient with this disease, therefore, requires about double the amount of food he would ordinarily need, in order to maintain his body weight, and if this demand is not met, the lack is made up by oxidation of his own body tissues.

C. P. Howard (2) sums up the clinical syndrome on this phase very well. He says, "Appetite is variable, although anorexia is uncommon except when the disease is running an unfavorable course. There is more often a bulimia or a voracious appetite with an especial craving for carbohydrate food." (That this is physiological there is now fair evidence to offer, for carbohydrates form a ready fuel and make the least drain on the already taxed iodine reserves of the body. Proteins deplete the iodine reserve and fats most of all, so it must be borne in mind that the goitre of iodine starvation may be due to either the depletion of iodine or to the demands made upon the over-taxed gland by foods requiring a great deal of it for the metabolic process (1).) "Again the appetite may be capricious and the patients, as in pregnancy, have longings for unusual kinds of food. The bulimia is said to occur in two forms—in one the craving occurs at long intervals in paroxysms extending over several days; in the other the hunger occurs almost daily for periods of an hour or so, though the bulimia in this variety is not as intense as in the former case." "Both types may accompany the diarrhea and vomiting but not necessarily so." Thirst, too, may be increased owing to the excessive "sweating."

Kunde, in her extensive work on hyperthyroidism in dogs, has consistently noted a voracious appetite which developed soon after the onset of the canine hyperthyroid symptoms or syndrome. She reports that such animals will eat, per diem, two or three times the food required by the normal animal. Lorenzi (3) states that thyroid feeding decreased the

normal emptying time of the stomach, in dogs, by two hours and forty-five minutes.

With the foregoing clinical and laboratory picture in mind we have attempted, in this investigation, to determine whether this desire for food was due to a desire for the taste and sensation of food ingestion (appetite), or whether it was due to an actual increase in hunger contractions over that of the control periods (the supposed objective criterion of true hunger).

EXPERIMENTAL METHODS. Five dogs were used throughout the course of the investigation, which extended over a period of nine months. Of the five animals originally used, two were again run on a much higher dosage after an interim of five months, during which time they were used for other work.

Due to the fact that our dogs were to be studied over a long period of time, it was found necessary to devise a rather different type of operation for gastric fistulae which would not enlarge. A short description of the technic is included in this paper.

The initial incision, not more than one inch long, is made half way between the umbilicus and the xyphoid process, one-half inch to the left of the linea alba and at right angles to it. The various layers of muscle are picked up en masse by hemostats to the left and right of the incision, and the same procedure is carried out with the peritoneum. One forefinger and a long-bitted, rubber tipped hemostat are introduced into the peritoneal cavity through the incision, the stomach palpated for the desired location of the fistula (fundus), and this portion picked up by the hemostat grasped in the other hand. Two retaining sutures one quarter inch apart are made through the exposed bit of stomach which is henceforth, for the period of the operation, to be supported by them. The exposed bit of stomach is no larger than the tip of the little finger. The serosa is sutured to the peritoneum by four stitches, the two end ligatures being from peritoneum to serosa to peritoneum. The same procedure follows with muscle and muscularis. The skin is sutured up to the fistular opening but not to the stomach layers themselves. Holding the retaining sutures up and apart, the incision through the walls of the stomach is made at right angles to them, and the gastrostomy tube (which is projecting from the bandage) inserted into the opening. After the tube and attached bandage are in place, the retaining sutures are pulled out.

The small cross skin incision is made to eliminate gastric juice erosion during healing. This usually occurs along the line of sutures. It is made laterally to utilize the transverse, rectus and oblique muscles as valves. The double retaining sutures simplify the insertion of the tube and make it possible to utilize a small portion of the stomach. The skin is never sutured to the mucosa of the stomach because this increases the tendency of the fistula to enlarge. Such fistulae rarely leak and may be dressed only twice at three day intervals. The dog is then ready for the experiment. A fistula made in the foregoing manner will be found quite satisfactory whether subsequent experiments extend over a period of a single week or many months.

The diet used for our dogs was a standard one, consisting of 200 grams of cooked, ground round steak mixed with 200 grams of bread and 200 cc.

of milk. This was maintained throughout the course of the various experiments, during control and induced hyperthyroid periods. When the desiccated thyroid was fed, it was mixed thoroughly with the food.

Twelve hour hunger contractions were observed, due to the fact that twenty-four contractions caused such a large excursion of the manometer float that if, as we expected, desiccated thyroid would increase them, we would have no means of observing the increase.

In order to establish adequate controls on our dogs, we ran daily graphs, on smoked drums, over a period of at least two weeks before and after our experiments. The experiments themselves extended over periods of from two weeks to a month, depending upon the time necessary for the individual dog to develop known symptoms of hyperthyroidism. Our criteria of the condition consisted of a steady falling off of body weight, resumption of the normal (or abnormal) desire for food, increase in both pulse rate and respiration, rise in temperature, restlessness and nervousness, hot and flushed skin, and falling hair. Daily records of these items were kept on each dog as an index of its condition.

Thirty minute drums were used to graph our final daily records. The drums were set in motion only when we had observed, by the excursion of the water manometer float on the drum, that an active period of the stomach was in course. The active period graphed, was taken as close, in point of time, to our standard twelve hours as it could be. The dogs were placed on the tables and the balloons blown up well before the time came to run them, one-half to three-quarters of an hour, so that we might observe both active and passive periods before taking the record for that day. We took, as well, many graphic records of runs of an hour to four hours, in order to determine the calibre of hunger contractions elicited over a longer period of time, on various animals, and came to the conclusion that there was no appreciable difference in our standard records and those of longer periods of time, and that hunger contractions, when once elicited, using the described technic, remained of the same character and strength over the 10 to 14 hour period chosen for our work.

In order that the conditions of the experiment remain as nearly constant as possible, as far as stomach tonus was concerned, we blew up and deflated the balloons several times before inflating them for the experiment. We then manipulated the dog's abdomen to destroy any high tonus which might have been present, and having done this, the balloons were inflated until the manometers showed a pressure of approximately four centimeters. This initial pressure was constant and standard and a line traced around the drum at this level constituted our baseline.

Results. In order to interpret our results more carefully, we employed the following method. The height of contraction and the distance from the baseline were measured on the last fifteen to twenty contractions on

each graph for the individual dog. These figures so gained were averaged and plotted on graph paper as the daily record for that dog. We likewise graphed the temperature, respiration, pulse rate and body weight.

As previously stated, five dogs were used to complete the experiment. Positive results were obtained (increase in hunger contractions) on four animals and doubtful results on the fifth. This latter animal appeared to be extremely afraid of the routine work, hence it is quite possible that the inhibition as result of apprehension which he betrayed was responsible for the doubtful results noted.

On the advice of Dr. Meta Kunde we adopted a minimal dosage of 2.5 grams of desiccated thyroid, per diem, for the average 10 to 11 kilo dog. This amount we found sufficient to develop symptoms of hyperthyroidism, including increased hunger contractions.

With the administration of desiccated thyroid in 2.5 gram dosage, and after a control run of at least two weeks, we noted a decrease in the height of hunger contractions and a lessened desire for food which lasted from three to eight days which gave way in turn to the resumption of a greater avidity for food and an increase in the height of hunger contractions. When the medication was dropped from the diet, the hunger contractions showed an enormous increase for a day or so with a subsequent daily tapering off toward the normal.

A 5 gram dosage was then administered to three dogs after control periods. The results here differed radically from the above. In one case we noted first a lessened intake of food, fasting for four to seven days (concurrent with depression of hunger contractions), then a period of abnormal hunger which lasted from two to four days, then a period of normal food intake. As soon as the medication was removed we again noted the hunger with a daily tapering off toward the normal. In this dog the hunger contractions as registered on our kymograph formed an index of the animal's condition. For instance, the real hunger periods (in most cases) were checked by the enormous excursions of the manometer float, which forced the water out over the top of the U-tube. The second dog of this run died during the course of this experiment after showing a rapid loss of body weight for three days. Other than an easy fatigue on the last two days, he appeared quite unaffected by any disease, respiratory or otherwise, until his sudden death. The autopsy showed normal viscera. The third dog of this series gave negative results throughout on both 2.5 and 5 gram dosage.

It is necessary to mention at this point that while as a rule the height and frequency of hunger contractions ran parallel with the hunger displayed by the individual dog, we have on many occasions noted an increased desire for food in dogs which displayed low tonus and low contractions when run on our daily experiment. Conversely, we have noted high hunger contractions in dogs which ate quite daintily when fed.

In an attempt to demonstrate the voracious hunger alluded to in the opening paragraphs, it was decided to run two dogs on a dosage of 15 grams of desiccated thyroid a day. Two of the original group were used, dogs in which we had already demonstrated an increase in hunger contractions under thyroid medication some months previously. An increase in hunger contractions was again noted but the true polyphagia sought did not appear until the 21st day of the experiment. It seemed to occur in both animals simultaneously even though one had shown consistently a greater desire for food than the other. From this time on to the 28th day, when the desiccated thyroid was dropped, the animals were very eager for food. In spite of these findings, however, we noted no consistent increase in the hunger contractions. The medication was then dropped from the diet and a control record taken. The initial sluggishness toward food and other symptoms were noted here as on the daily 2.5 gram dosage, but the enormous contractions noted after starting the 5 gram medication were not seen. During this experiment the dogs exhibited an increased susceptibility to mange, which was hard to clear up with the most stringent measures. One of the dogs evinced a desire to eat its own feces.

Discussion. During nine months of the current year, we have, in various experiments, observed hunger contractions on twelve dogs. A part of this number was run on twelve hour experiments and the remainder on twenty-four hour experiments. The twenty-four hour animals were fed immediately after running the experiments in order to prepare them for the next day, and the twelve hour animals twelve hours later for the same reason. We have repeatedly seen our twelve animals give high hunger contractions on their daily record and yet give no evidence of hunger when fed twelve hours later when, presumably, their hunger should be much greater. We have also noted this phenomenon in our twenty-four hour dogs, which were, as we have said, fed immediately after their run. Conversely, we have noted experiments in which the animals have displayed little more than a low tonus record, and yet, when fed, manifested a great desire for food. While this latter finding was general for both groups of dogs, it was only significant in the case of the twenty-four hour animals.

We must first assume that the blood of a given animal is exhausted of its nutrient constituents, due to starvation. We have then conceived of a condition which may, in some manner, cause the parenchymatous and general body cells to elaborate certain substances (metabolites) whose passage through the arterial system of the stomach so acts upon specialized receptor cells that hunger contractions are elicited in greater or less degree, according to the degree of stimulus. Or it may be that these are preformed elements, already present in the blood, whose rôle is only played when the nutrient constituents are exhausted (that there are metabolites we have fair evidence (4)). In the latter case the lack of food materials may lower the

threshold of irritability of the receptor cells; concurrently with the gastric stimulation, we must assume stimulation of that area of the central nervous system which gives rise to the consciousness of hunger or a "sharpening of appetite." It is the increased tone or irritability of this center during hunger that makes our perception of olfactory or gustatory impulses so much keener.

If we grant, then, two such places of metabolic action, as suggested above, we can readily perceive that if a toxic substance is present in the stomach, which deadens the perceptibility of, or raises the threshold of, irritability of the receptor cells to the circulating metabolites, we may have the sensation of hunger without there being actual hunger contractions, by reason of the other rôle of stimulation of the central nervous system.

Lack of hunger with powerful hunger contractions may be accounted for in the following manner: If the sharpening of appetite does not occur due to the depression of the central nervous system by toxic substances, etc., the hunger contractions are interpreted as gastric distress or nausea, without desire to eat. It is possible also that gastric contractions may be called forth by local stimulants. In such circumstances the failure of the metabolites to stimulate the hunger center would cause the gastric sensations to be interpreted as pain.

Hunger contractions are inhibited when food is taken into the mouth. According to present interpretation, the hunger sense should disappear at this juncture—if hunger contractions are the only factor. We do not believe that this is the case, as one may readily ascertain by the simple experiment on himself.

Though our dogs on the smaller dosage did not show any great desire for food, they ate all that was given to them and showed a decrease in body weight, hence it is possible that they would eat far more if fed. The bulimia that they exhibited may be analogous to the second type of hunger described by Howard (2) and alluded to in the opening paragraphs.

The writers take this opportunity to express their sincere appreciation for the kindly help and criticism tendered by Dr. Arno B. Luckhardt.

CONCLUSIONS

1. A small dosage of desiccated thyroid sufficient to produce the syndrome of hyperthyroidism in dogs causes an increase in the height of hunger contractions.

2. There seem to be two effects produced by feeding desiccated thyroid; one in which a hunger producing constituent causes an increase in hunger and the other in which a toxic, depressant constituent may or may not overbalance the former, depending upon the degree of tolerance to the latter that the individual dog may develop. Or, it may be that we are

dealing with one and the same substance, which depends upon the tolerance developed by the individual dog to show its hunger effects.

3. In the 15 gram dosage, the increase in hunger effect seems to overbalance the toxic effect although we may have noted the same result in the 5 gram dogs had we run them long enough.

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THE EFFECT OF CALCIUM AND MAGNESIUM IONS ON THE GASTRIC HUNGER CONTRACTIONS

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The antagonism between Ca and Mg ions has long been the subject of investigation by biologists. This paper presents further studies on this alleged antagonism, namely, the effects of these ions on the contractions of the empty stomach (hunger contractions).

Meltzer and Auer obtained complete anesthesia upon subcutaneous injection of magnesium salts (1), and showed later (2) that this state could be alleviated by injections of calcium salts. In further studies on the properties of Mg salts they noted that these salts inhibited peristalsis of the gastro-intestinal tract (3). This has been amply confirmed by Bancroft (4) and others.

MacCallum (5) noted that calcium produced an inhibitory effect on intestinal peristalsis. It is known also that increase of either calcium or magnesium ions depresses muscular and nervous structures but nevertheless the effects of Mg may be removed by the addition of Ca (6).

Luckhardt, Brannon and Waud (7), working on thyreoparathyroidectomized dogs noted that their animals, which had been fed 20 grams of $MgCl_2$ daily, became very sluggish after a week of such medication, a condition which bordered on a state of anesthesia. These animals would be dormant and would move only upon urging. In addition these dogs refused to eat the food offered them. If, however, calcium salts, notably the chloride and lactate, were administered per os in dosages of 1.5 gram per kilogram of body weight, these animals became active and would eat voraciously within two hours after administration with an appetite bordering on a polyphagia or bulimia. Their observations suggested the problem at hand, namely, the effects of Mg and Ca on the gastric hunger contractions.

METHODS AND RESULTS. 1. *Oral administration.* A number of dogs were prepared with gastrostomies using the technique described by us in another paper. To these animals was fed a daily diet consisting of 200 grams of ground, cooked round steak mixed with 200 grams of bread and 200 cc. of milk. The weights of these dogs were followed daily to determine whether or not the diet was adequate. We recorded the hunger

contractions at twenty-four hour intervals, the animals being fed immediately after records had been taken. Control records were taken for periods of two weeks before and after administration of Ca and Mg salts. During the experimentation period, 20 grams of magnesium chloride per os (determined to be the minimal dose having the greatest constant effect) were administered twelve hours previous to the daily routine of recording the hunger contractions. A return to normal was allowed (cessation of Mg administration) and another period of magnesium feeding was then again instituted. This period was followed by the administration of calcium lactate. The amount administered was 1.5 gram per kilo of body weight given two hours previous to the registration of the hunger contractions (8). Calcium lactate was then substituted for the Mg and its effect on the hunger contractions was noted. The balloon method of recording gastric contractions was used throughout.

We recorded our results in the following manner: The height of contractions and the distance from the baseline (4 cm. H_2O pressure) were measured on the last fifteen to twenty contractions of each group for the individual dog on each day. These figures were averaged and plotted on graph paper as the average daily record for that dog. Daily observations on temperature, respiration and pulse rate were recorded but these latter observations showed nothing significant during the course of the experiment. A comparison of the height of normal contractions to that of the control period of experimentation would represent quantitatively the results obtained. At various times during the control or experimental periods continuous records of from one hour to four hours were taken. Five dogs were used in this group of experiments.

Following administration of $MgCl_2$ a marked decrease in the height of the contractions was noted in every animal during the period of experimentation. Occasional periods of high hunger contractions, some lasting 2 to 3 days, were noted to break through the period of depression. Following removal of magnesium from the diet there was a return to the original high normal contractions with an occasional low period of contraction after the first day, indicative of a depression outlasting the actual period of administration of the magnesium salt.

Calcium lactate given by mouth to normal dogs had no effect on the height of the gastric contractions displayed by the animals.

Calcium lactate administration, following magnesium medication, (Ca given two hours previous to taking a record) in most cases diminished the gastric depression; for the hunger contractions were notably higher. On the day following, if a normal were to be resumed, we noted again the low hunger contractions indicating, as did the previously reported observations, a depressive effect of the magnesium medication but the return to normal took less time. In a series of experiments in which calcium was

administered every four to five days, during a long period of magnesium medication, high hunger contractions were observed in most cases on the days that calcium was administered. Another interesting observation was that in some cases the period of high hunger contractions in this latter group of experiments followed calcium administration in approximately two hours. It is interesting to note that blood calcium, according to Hjort (8), is highest two hours after administration per os. This latter statement confirms Luckhardt's observation that parathyroid tetany symptoms are usually alleviated completely two hours after administration of calcium by mouth.

In this series of experiments we observed that while as a general rule the height and frequency of hunger contractions could be taken as an index of the hunger displayed by an individual dog, we have on many occasions noted desire for food in dogs which displayed low gastric tonus and low contractions. Conversely, we have noted periods of high hunger contractions, during the period of magnesium medication, in which the animals ate less avidly of the food set before then. Care was taken to see that these animals had sufficient water at all times.

2. *Intravenous injections.* Five dogs were used in this series of experiments. The method used in recording the effects of the various injections of the hunger contractions was as in the previous group of experiments. The animal was allowed to lie quietly on the table and a normal record of the contractions of the empty stomach was taken. The hind leg was then gently constricted to facilitate injection into the saphenous vein. Injections were made on various days of equal volumes of distilled water, Ringer's solution, physiological saline, magnesium chloride, and calcium chloride solutions, respectively, and the results noted.

The effects of magnesium injections were continuously recorded for a period of one and one-half hours following the injection. Calcium injections were made and results noted over a similar period of time. The calcium content of the solutions used was equimolecularly equivalent to the magnesium concentrations used in the previous experiment. In some instances calcium injections were given shortly after the magnesium injection to conclude this group of experiments.

The animals used in these experiments were well trained from the previous experiments, which probably accounts for the fact that manipulation of the leg and insertion of the needle into the vein caused no inhibition of the gastric contractions.

Injections of distilled water, Ringer's solution and physiological saline in the quantities used (10 cc.) had no demonstrable effect on hunger contractions. Magnesium injections in all concentrations which displayed any effect whatsoever caused an immediate cessation of hunger contractions and loss of tone. Shortly after injection there ensued violent vomit-

ing that lasted for several minutes. The duration of the former effect was dependent entirely on the concentration of the magnesium salt, although in every case cessation of hunger contractions and loss of tone were noted. The amounts injected varied from 100 mgm. per kilo of body weight to 50 mgm. per kilo. Calcium injections had the same notable effect as did the magnesium injections—immediate cessation of hunger contractions with loss of tone. Very small doses, 2.75 mgm. per kilo to 1.25 mgm. per kilo, had no effect on hunger contractions or loss of tone, but vomiting was observed with quantities as low as 2.75 mgm. per kilo.

Calcium following magnesium injection had no effect except to prolong the period of depression.

3. *Bathing gastric strips with Mg and Ca salts.* Gastric strips with the mucosa stripped off were taken from the pyloric end of the stomach. They were cut approximately 1 cm. by 4 cm. in size and suspended in a muscle chamber. By means of a three-way stopcock, magnesium, calcium and Locke's solutions were run into the chamber by displaced air pressure from a bottle into which regulated drops of water were falling by siphonage. The solutions and muscle chamber were kept in a water bath at 37.5°C. Oxygen was allowed to bubble through the fluid in the muscle chamber. Regular contractions could be best obtained from strips that had been allowed to remain in the ice box at 3°C. for 24 hours covered with Ringer's solution. Normal contractions were recorded for a period of time, the strip being bathed in a slowly running Locke's solution. Calcium chloride (0.05 per cent solution in Locke's) or equimolecular magnesium sulphate was then run into the chamber rapidly and when enough fluid had run out through the overflow tube to insure that the solution had been completely changed, the rate was slowed so as to conserve the solution.

The sequence of solutions on any gastric muscle strip was usually as follows:

1. Bathing with Locke's (controls).
2. Magnesium following Locke's.
3. Locke's solution (resumption of normal).
4. Calcium bathing.
5. Locke's solution (resumption of normal).
6. Magnesium followed shortly by calcium.
7. Locke's.
8. Calcium + magnesium in equal quantities.

Magnesium invariably produced a depression with complete obliteration of contractions and loss of tone. Only on a few occasions did an actual contraction break through.

Calcium, on the other hand, invariably caused an increase in tonicity and rhythmicity of the contractions which became more uniform in size

and increased in rate. Calcium following a period of magnesium depression caused a return of the contractions, in most cases at a time less than the return caused by bathing with Locke's solution.

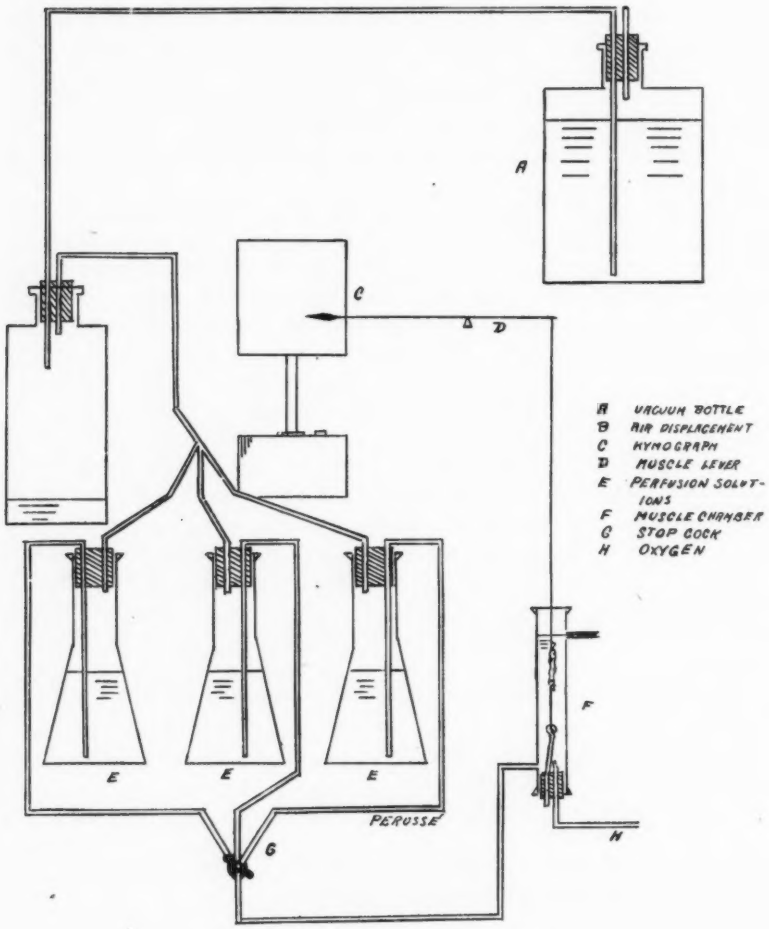

Fig. 1. Apparatus for gastric strip work

Bathing with a mixture of calcium and magnesium had a primary effect of slowing the contractions slightly and then increasing the rhythmicity and tonicity following the short period of slow contractions.

DISCUSSION. As has already been mentioned, we have observed on many occasions *desire* for food in dogs which displayed low contractions on a low tonus wave, and, conversely, we have noted periods of high hunger contractions in which the animals ate sparingly of the food set before them.

The effects of the intravenous injections of calcium and magnesium have been entirely central—stimulation of the vomiting centers with accompanying inhibition of gastric motility through impulses via the splanchnic. Direct or peripheral effects may be thus masked. This could be tried on animals with a denervated gut. Danielopolu (9) claims to have noted increased hunger contractions on a man with a gastrostomy after intravenous injection of 10 cgr. of $CaCl_2$, and inhibition of hunger contractions with doses of 15–25 cgr. or greater. Assuming that his subject was of average size and weighed 65 kilos—the dose to increase hunger contractions would be 1.54 mgm. per kilo of body weight. We have tried doses of as low as 1.25 mgm. per kilo of body weight on the dog and have noted no increase of hunger contractions. In his larger doses (3 mgm. per kilo) we explain the inhibition as due to central effects, as we have obtained vomiting with as small a dose as 2.75 mgm. per kilo.

The effects of magnesium on the gastric strips are on the neuro-muscular mechanism itself and the antagonism between the magnesium and calcium is marked. That calcium is stimulating to the gastric strip and magnesium depressing has also been shown. Neutralization of one ion by the other in the simultaneous bathing in the two solution mixture again shows the antagonism between the two ions.

The writers wish to express their sincere appreciation for the kind help and criticism tendered them by Dr. Arno B. Luckhardt.

CONCLUSIONS

1. Magnesium salts given orally cause an inhibition of hunger contractions that can be relieved by giving calcium salts. Calcium administration per se has no effect on hunger contractions.

2. Central effects caused by injections of magnesium and calcium salts may mask any true effect that these salts may have on hunger contractions. Cessation of hunger contractions and loss of tone were the effects observed. Very small doses of calcium have no effect on hunger contractions. A calcium injection following on magnesium injection does not diminish the depressive action of the magnesium on the hunger contractions.

3. Magnesium depresses rhythmical contraction of gastric muscle strips. Calcium enhances gastric strip contraction. Calcium after magnesium depression allows contractions to return to normal more quickly than does

bathing with the original Locke's solution. In bathing with calcium and magnesium solutions simultaneously, the calcium effects predominate.

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ON THE RECOVERY OF THE HEART IN ELECTRIC SHOCK[1]

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The fatal outcome of electric accidents may be due to involvement of the respiratory center or of the heart or of both (1). The effect upon the respiratory center is inhibition or paralysis. Theoretically the heart may likewise be inhibited or paralysed but by far the most common effect is ventricular fibrillation with which the present paper is concerned. In general it is believed that the high voltage of power plants and transmission lines predisposes to respiratory paralysis while the low voltage of house currents results in ventricular fibrillation, although no sharp distinction can be made because of the variable factor of the contact made and consequently of the amount of current which passes through the body.

A competent observer could distinguish between the common respiratory and cardiac effects if he were present shortly after the accident. If the respiratory center alone is affected respiratory movements are absent but the heart continues to beat and, with adequate artificial respiration to tide over the period of paralysis, recovery may follow. On the other hand, if the heart alone is affected no arterial pulse or heart sounds are present although a venous pulse may be temporarily visible in the neck because auricular activity does not always cease coincident with the onset of ventricular fibrillation. In this case respiratory movements continue until asphyxiation, due to failure of the circulation, is complete. Artificial respiration is of no avail under these circumstances because, regardless of the aeration of blood in the lungs, the failure of the heart to activate the circulation inevitably results in death. When both respiratory paralysis and ventricular fibrillation result no sign of life will be observed unless auricular activity persists with resultant jugular pulsations.

It will be noted that the phenomenon under discussion involves the two usual criteria of life, cessation of respiration and absence of arterial pulse. Since however individuals have been revived, at least after a period of suspended respiration, the condition has been regarded as one of suspended animation (2), and D'Halluin (3) has particularly emphasized the

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uncertainty of an exact definition of death as regards the organism as a whole and also as regards the component parts. Kuliabko (4) revived the human heart by artificial circulation twenty hours after death and Drinker (5) believes recovery from drowning to be possible twelve to fifteen minutes after submergence. The so-called vital organs can thus recover function after remarkably long periods of inanimation. The chances of such recovery are of course greater if animation ceases suddenly as in the case of accidents. Doubtless this statement would not hold true after death from disease when the vitality of the organism has been seriously depleted.

In the present investigation one experiment (April 2, 1928) was performed in which normal heart action was apparently recovered in a dog after twenty minutes of fibrillation but little stress is laid on such exceptional cases although, as will be shown, ten minutes of inanimation is not incompatible with complete recovery.

Similarly Crile and Dolley (6) succeeded in reviving dogs, apparently dead from over-doses of chloroform and ether and from asphyxiation, after ten minutes of inanimation and they were able to revive the heart temporarily twenty-three and thirty-five minutes after apparent death.

This evidence supports the possibility that experimental animals and *ipso facto* human beings, made inanimate by certain accidents, may be revived after a considerable period of suspended animation. In the present research several dogs, whose hearts were left in fibrillation for five minutes, were permanently recovered and remained in good health. This point is of supreme practical importance since, under favorable circumstances, it should be possible in this time to bring to bear remedial measures of demonstrated efficacy.

The outstanding feature in electric shock is ventricular fibrillation. "In many primary heart failure is undoubtedly the commonest mode of death by electric currents" (Jex-Blake). Williams, who has recently had opportunity to make electro-cardiographic observations on a number of electrocuted criminals, states that most of his electrocardiograms show ventricular fibrillation; in some of these the fibrillation persists while in others it gives place to coördinated beats which are slow, irregular and atypical.

Hoffa and Ludwig (7), in 1850, were the first to show that electrical stimulation of the mammalian heart causes fibrillation. If the auricles are stimulated with a tetanic current the fibrillation continues only so long as the stimulus is applied. The ventricles (of the dog), on the other hand, when once in fibrillation ordinarily continue to fibrillate until death intervenes.

While electrical stimulation is the simplest method with which to establish ventricular fibrillation it may also be induced by pricking Kronecker's point in the anterior auricular-ventricular groove and by coronary liga-

tion (8). These and other observations have led to the development of a number of theories to explain ventricular fibrillation. They have been sympathetically discussed by Gallavardin (9).

Provost and Battelli (10) found that an *alternating current* of 10 to 15 volts was sufficient to fibrillate the dog's ventricles while a considerably stronger *direct current* (50 to 550 volts) was required to produce the same effect. They also state that it is possible to stop fibrillation of the dog's heart by counter-shock. In their experiments they found that if the heart was fibrillated with 40 volts the fibrillation could be stopped by the prompt application of a current of 240 to 4800 volts. This idea that counter-shock of some kind is beneficial in fibrillation is prevalent in the literature (11) presumably on the ground that a stimulus sufficient to throw all the muscle fibres of the heart into action at once will make them, after the refractory period is passed, susceptible to an orderly response initiated by the conducting system.

EXPERIMENTAL. The present investigation represents a frank effort to find a method theoretically applicable to accidents in which the human heart is fibrillated as the result of contact with electric circuits. It was subsidized and encouraged by a Committee (12) created to study the scientific aspects of electric shock to the end that practical means might be found to reduce the number of fatalities which occur as the result of the increased use of electricity. It is not presumed that the conclusions drawn from these experiments warrant a direct and immediate application to man but it is believed that they are sufficient to warrant further experimental perfection for this purpose. Certainly it can be asserted that there is theoretical possibility of saving lives which, according to present practice, are obviously doomed. Results which may be of help in approximating the fundamental problem of fibrillation are only incidental in this paper.

Because the dog is the best available experimental animal for such studies, in that this heart practically never spontaneously recovers from ventricular fibrillation, these animals were used exclusively. They were always fully anesthetized and, when it was intended to save them for observation subsequent to the experimental procedure, asepsis was carefully observed. One of these animals, which was "dead" for over eight minutes, is at present a pet in the author's home.

Special characteristics of ventricular fibrillation. Whatever may be the basic upset which leads to fibrillation the phenomenon is certainly different in auricles and ventricles. Auricular fibrillation is recognized clinically without involvement of the ventricles and when ventricular fibrillation is produced experimentally the auricles may continue to beat in a normal rhythm. Thus in one experiment (Oct. 28, 1928) a dog heart, isolated on coronary perfusion, was fibrillated by electrical stimulation. The auricles

continued rhythmic beats while the ventricles remained in fibrillation for two hours at the end of which time a few cubic centimeters of strong potassium chloride were introduced into the perfusing cannula. Both auricles and ventricles promptly came to rest. The perfusion being continuous, the excess potassium was shortly washed out and the auricles began to beat followed in a moment by a coördinated ventricular rhythm.

This experiment emphasized the difference in response of the auricles and ventricles and, incidentally, the remarkable efficacy of potassium chloride in stopping fibrillation. Furthermore when stimulating electrodes are applied to the auricles these structures exhibit fibrillation only so long as the current is applied, regardless of its strength. A similar stimulation of the ventricles, on the other hand, will establish a condition in which the fibrillation continues indefinitely after the stimulus is removed.

On the ventricular mass itself a stimulus is effective regardless of the amount of tissue traversed. On October 28, 1927, a tetanic induced current was used. With one electrode on the right and the other on the left ventricle separated 2.4 cm. permanent fibrillation was not induced until the secondary coil was brought to 6. Coronary perfusion was then begun and a normal rhythm recovered by introducing 1 cc. of 2.5 per cent KCl into the cannula. With the electrodes similarly placed the same strength of current was required to establish fibrillation. The position of the electrodes was then changed so that they were 0.2 cm. apart and the requisite stimulus determined. Permanent fibrillation was set up when the secondary coil was moved into the former place, i.e., 6 on the scale.

In another experiment, October 31, 1927, the direct current was used. With the electrodes 2.8 cm. apart a current of 9.5 volts and 12 to 17 milliamperes was required for fibrillation. Reversal of the current gave the same result.

These observations define the electrical disturbance requisite for fibrillation and indicate that it is not the amount of tissue involved but rather the intensity of the disturbance which is the determining factor. This disturbance spreads quickly from a sharply localized point and involves the whole muscle mass. The difference in response of auricles and ventricles offers another interesting problem. It appears probable that there is no structural difference in the muscle fibres to account for this. On the other hand the ventricles are characterized by the conducting system and by the complicated overlying of sheets of muscle. Vagus stimulation, although it rests the auricles, is quite ineffective to stop ventricular fibrillation (Oct. 17, 18, Nov. 11, 1927) and fibrillation of the ventricles is readily induced during vagal inhibition (Nov. 9, 1927). This suggests that the disturbance is in or distal to the conducting system. The remarkable rapidity with which the fibrillation spreads from the locus of irritation and the phenomenon of the Kronecker Puncture accords with the idea that the

conducting system is primarily involved. Analysis of the effect of ligation of the coronary arteries, which induces fibrillation, might illuminate this point because it might thereby be possible to set up localized fibrillation, the existence of which would argue against the spread of disordered impulse from muscle bundle to muscle bundle. Such a demonstration would not preclude the concept, originally suggested by Gotch, that the fundamental condition is associated with local ionic dissociations; it would merely define them to the conducting fibres or nodal tissue.

In concluding this section it is desirable to point out that in but one dog used in this series of experiments was it impossible to establish permanent ventricular fibrillation. This was a very young female puppy (May 18, 1928) in which 110 volts were repeatedly applied directly to the ventricles for five-second periods. Each time the ventricles recovered spontaneously after remaining in fibrillation for about a minute. This accords with the experience of other observers that an occasional dog, usually a puppy, is found which does not succumb to ventricular fibrillation.

Effect of cold. Superficially fibrillation appears to be a condition of hyper-irritability of the tissues. If this were so, chilling should reduce the irritability and benefit the condition. On November 23, 1927, the introduction of 11 cc. of ice-cold 0.9 per cent NaCl into the perfusion cannula of an isolated heart which was in fibrillation appeared to stop fibrillation but the preparation was not in good condition because the perfusion was delayed due to a fault in the pressure system and because the temperature of the perfusate was too high. The experiment was repeated, October 17, 1928, on an intact animal, the cold solution being injected into the central end of the carotid under heavy pressure. In this case cold was without effect as it was in a further experiment, October 10, 1928, in which the fibrillating ventricles were thoroughly chilled with an ethyl chloride spray.

Conducting system extracts. De Moor (13) and Haberlandt (14) have published results which lead them to the hypothesis that the conducting system liberates a hormone essential to coördinated cardiac activity. Their experiments rest on the exhibition of saline extracts of the conducting system in stimulating and coördinating activity in the isolated heart and it is proposed that the substance will be of benefit in ventricular fibrillation.

To test this possibility two experiments were performed (Nov. 14, 23, 1928) on the perfused and fibrillating heart. The extracts were prepared as follows (De Moor): the conducting system, including the sinoauricular node, was carefully dissected out of a fresh beef heart brought to the laboratory while still warm, ground in a mortar with 12 to 15 cc. 0.9 per cent NaCl, stirred continuously for three hours and centrifugalized to obtain a clear supernatant fluid. This fluid was injected into the per-

fusion cannula, 5 to 7 cc., at a time without the slightest apparent effect upon the fibrillation.

Because it has been suggested that the active principle in these extracts is an acetyl-chlorine and because acetyl-chlorine produces a vagus-like effect (15) several similar tests were run with acetyl-chlorine hydrobromide (Dec. 3, 7, 9, 1928). From 0.02 to 0.8 mgm. dissolved in 0.9 per cent NaCl was injected into the perfusion cannula of an isolated heart a total of twelve times. In every case the substance produced a pause of the auricles but was wholly without effect upon the ventricular fibrillation.

Drugs. A number of drugs have been suggested as of benefit in fibrillation and specifically of benefit in ventricular fibrillation. The pharmacopeia, however, offers few cardiac agents which are not stimulants. It is not surprising, therefore, that most of them should prove worse than useless in ventricular fibrillation. Since, however, the underlying cause of fibrillation is unknown it was clearly advisable to give these several agents a trial.

The outstanding member of this group is quinidine sulphate. Quinidine has been used successfully in auricular fibrillation. It is supposed to be of benefit by prolonging the refractory phase of auricular muscle and thereby putting a stop to the so-called circus movement (16). There is some evidence also that quinidine lengthens the refractory period in the dog's ventricles although Drury, Horsfall and Munly (17), who published this statement, also report that quinidine often contributed to the development of ventricular fibrillation. On the assumption that the basic trouble is the same in both auricular and ventricular fibrillation, i.e., a circus movement by which an impulse is transmitted round and round a muscle path in much the same manner that Mayer (18) found a contractile impulse could be made to travel in a Medusa ring, Levine and Fulton (19) have recently successfully used quinidine in several cases of ventricular tachycardia. It is to be pointed out, however, that even if their assumptions are correct there is a fundamental difference between tachycardia and fibrillation and that a drug which might benefit the one would not necessarily benefit the other. It is true that fibrillation induced by electrical stimulation appears to be preceded by a brief period of tachycardia. But quite the contrary is true of fibrillation induced by other causes, i.e., a preponderance of calcium chloride in the coronary perfusate, as has been repeatedly demonstrated. In the latter case the fibrillation is preceded by a number of slow, powerful contractions. It seems probable, therefore, that the tachycardia of electrical stimulation bears no direct relation to the subsequent fibrillation.

Quinidine was tried twice in the left ventricular cavity of the fibrillating heart (Feb. 17, 20, 1928) without effect. This result might have been expected since absorption through the endocardium is very slow. It was

also tried four times in the coronary circulation of the isolated and fibrillating heart. Once it appeared to be of benefit, twice the effect was uncertain or bad and once it accentuated the fibrillation (Feb. 16, Nov. 22, 1928). The difficulties of accurate dosage by this procedure are obvious and no great stress is laid on these results but the general impression left on the observer was that quinidine is actually deleterious.

Other drugs—caffein-sodium benzoate, cardiozol, coraline, coramine, digatilin, ouabain—reputed to have a cardiac action, were either without effect or accentuated the fibrillation. As in the case of quinidine they were convincingly tested in the coronary circulation of the isolated heart and in different doses. Since they were all either negative or disadvantageous in effect it is needless to detail any of the experiments.

MacWilliam (20) has reported recovery of the fibrillating cat's ventricles by the intravenous or intraventricular use of certain other substances when accompanied by cardiac massage. These are urethane, strontium chloride, adrenalin, hirudin and pilocarpine. The first three of these were tried in the present experiments with unsatisfactory results which need not be dwelt upon. There seemed no sufficient reason to trouble to try the last two. The cat's heart is an unsatisfactory preparation upon which to study fibrillation because of its tendency to spontaneous recovery and D'Halluin has emphasized the inherent dangers of cardiac massage. Consequently there seemed little or no hope of practical ends in repeating MacWilliam's experiments further.

In addition to the substances thus far enumerated there remain two which are at least of theoretical promise: magnesium chloride because of its depressant action as shown by Meltzer (21), and rubidium chloride because of its kinship to potassium chloride and because Roffo and Lopez-Ramierez (22) have reported experiments purporting to show that it is beneficial in auricular fibrillation.

Magnesium chloride was tried on one perfused and fibrillating heart (Jan. 5, 1928). The injection was made into the cannula as usual, 10 cc. of a 1 per cent solution being used. No apparent effect was produced. Shortly thereafter 5 cc. of 1 per cent KCl, similarly introduced, promptly brought the heart to rest. After a brief pause an orderly coördinated rhythm was established.

Rubidium was tested as the carbonate (April 23, 1928) and as the chloride (Nov. 9, 1928), on the perfused and fibrillating heart. Five cubic centimeters of 1 per cent $RbCO_3$ accentuated the fibrillations. Evidence is at hand and will be reported elsewhere to show that this result may be explained by the action of the carbonate. When rubidium was used in the form of chloride, however, it was not as effective as potassium chloride although it did act in a strong solution. Thus when 5 to 6 cc. of 0.5 per cent KCl were used, the fibrillation stopped promptly, while 10 cc. of 0.5

per cent RbCl were without effect. Similarly 2 cc. of 2 per cent KCl stopped the fibrillation in two seconds while 3 cc. of 2 per cent RbCl did not stop the fibrillation until after nine seconds.

Significance of potassium chloride in controlling fibrillation. It has already been briefly noted that potassium chloride is capable of stopping ventricular fibrillation. This might be inferred from the potassium inhibition of the normally beating heart and was demonstrated by Hering (23) at the International Physiological Congress in Heidelberg. If an isolated heart be made to fibrillate by electrical stimulation it is a simple matter to recover a normal rhythm by introducing a few cubic centimeters of 1 to 2 per cent potassium chloride into the perfusion cannula. Under these circumstances the strong potassium is carried into the coronary arteries and produces a complete inhibition of the heart which is followed, as the excess of potassium is washed out by the normal perfusate, with a normal coördinated beat.

Evidence will be presented to show that this principle can be applied to the resuscitation of the fibrillating ventricles in the intact animal. It should, however, be emphasized that the potassium alone is insufficient for recovery. *It must be washed out* of the coronary bed with a calcium solution to counteract the potassium effect. This will become clear when the details of the method are presented.

D'Halluin, whose paper was brought to the writer's attention some time after this investigation was under way, also sought to make use of this action of potassium and of its antagonist, calcium, in the recovery of fibrillating hearts. Unfortunately he did not recognize the importance of the direct coronary route and sought to introduce the potassium into a vein and to facilitate its approach to the coronary bed by cardiac massage. This is a method, previously used by MacWilliam, which D'Halluin finally discarded as fraught with too much danger for use in man. Cardiac massage has a further disadvantage, as D'Hallium points out, in that it conduces to fibrillation.

With the efficacy of potassium chloride in the coronaries for the control of fibrillation thus established it was desirable to discover, with an eye to its possible application in man, if a simpler approach might be possible. Consequently it was tried in the pericardial sac, in the right auricle and in the left ventricle with essentially negative results as the following experiments indicate.

Potassium chloride in the pericardial sac. Eight cubic centimeters of 5 per cent KCl were introduced into the pericardial sac after fibrillation was established in one dog weighing 5.7 kilo (Dec. 17, 1927). It was wholly without effect.

Potassium chloride in the left ventricle. This procedure was followed in sixteen animals, the introduction into the ventricular chamber being made

with a hypodermic needle after the heart was in fibrillation. The amount of fluid used, usually 5 cc., was not significant since any in excess of the ventricular capacity overflowed through the aortic valves. The result was considered positive if the fibrillation stopped in two or three minutes after the injection. When the strength of potassium chloride used was 5 per cent or less the results were uniformly negative; with 15 per cent three positive and five negative results were obtained and with 20 per cent there was one positive result. In a single experiment (Dec. 1, 1927) 5 cc. of 5 per cent potassium chloride were introduced into each ventricle without benefit.

The foregoing is evidence that potassium chloride may be absorbed from the ventricular chamber. Further evidence is available from experiments on the perfused heart. With this preparation 10 to 20 per cent KCl introduced into the ventricle will usually stop fibrillation especially if the perfusion is discontinued during the period of absorption. In a heart so manipulated normal beats sometimes followed renewal of the artificial circulation. It was usually necessary, however, to wash out the excess potassium with normal saline, using a second ventricular needle for the purpose, to massage the heart (a mechanical means of emptying the ventricle) or to stimulate activity by introducing adrenalin into the perfusion cannula.

When the circulation is intact and the heart is in fibrillation potassium chloride of adequate strength introduced into the ventricle frequently coagulates the stagnant blood. Although the tendency to coagulation can be overcome by the use of heparin no means other than massage have been suggested for counteracting the potassium inhibition. Attempts to wash out the excess potassium with saline, which was effective with the isolated heart, were unsuccessful. While cardiac massage is theoretically applicable to man and has been successfully employed in the operating room it is not to be considered as a first-aid measure. Furthermore D'Halluin who used it in experimental electric shock in conjunction with the intravenous injection of potassium chloride, discarded it as fraught with far greater danger than benefit as an aid in ventricular fibrillation.

In five experiments effort was made to reactivate the intact heart brought to rest in this manner by following the potassium chloride with strong calcium chloride or with adrenalin or both. The results were quite negative.

In five other experiments rhythmic electric shocks were applied to the heart either directly or through the chest wall for the purpose of arousing activity in the potassium inhibited heart. Powerful induced make and break shocks and the direct house current up to forty volts were employed. In every case some ventricular response developed but this was mostly confined to the right ventricle or to an area in the A. V. groove but in no

case did the contractions continue to improve and sometimes the ventricles were thrown back into fibrillation.

The outcome of this series of experiments gave clear indication of the uselessness of depending upon ventricular absorption of potassium chloride in the control of fibrillation.

Potassium chloride in the right auricle was tried in four experiments. On October 18, 1927, 2.5 per cent KCl was introduced into the right auricle of a heart in fibrillation and into both right and left auricles of the same heart after an isolated circulation was established. On October 25, 1927, an isolated heart was fibrillated and a fine stream of 10 per cent KCl directed into the right auricle against the region of the sino-auricular node. On November 8, 1927, cotton soaked with 10 per cent KCl was held pressed against the nodal region of a fibrillating heart. On November 11, 1927, cotton soaked with a saturated solution of potassium chloride was similarly applied. Of these several efforts the latter only seemed of any benefit and even in this case the result was not outspoken.

In view of the impossibility of making practical use of the last method, which appeared to have some merit, no further similar trials were undertaken but the procedure is worthy of further study as a means of locating the fibrillary disturbance. If a crystal of potassium chloride, applied to the sino-auricular node, should bring the fibrillating ventricles to rest it would suggest that the basic upset is in the conducting system rather than in the muscle fibres.

Potassium chloride and calcium chloride in the central end of the carotid. It has been conclusively shown that 1, potassium is the best available agent for the control of fibrillation; 2, the most satisfactory method of administration is via the coronary arteries, and 3, if the excess potassium is washed out of the coronaries after a period of potassium inhibition, as in the perfused heart, a normal sequential beat is resumed.

The possibility of the application of this principle of resuscitation in an animal with the circulation intact was first successfully demonstrated December 19, 1927. Subsequently the technique was variously modified and the following procedure finally developed:

The dog is fully anesthetized with morphia and ether and the central end of the right carotid is cannulated. Moist electrodes are applied to shaved areas on either side of the thorax and the 110 volt direct house current led through them. Two seconds are ordinarily sufficient to throw the ventricles into permanent fibrillation. The solutions, warmed to body temperature and saturated with oxygen are injected into the carotid under a pressure of 150 mm. Hg. It is assumed that this head of pressure drives the stagnant blood beyond the aortic arch and forces some of the solution into the coronary arteries. In fact if the chest be opened and the heart exposed the organ can be seen to expand. The first solution consists

of 0.5 per cent KCl in 0.9 per cent NaCl to which 0.25 mgm. of heparin per cubic centimeter is added. The amount injected is about thirteen cubic centimeters per kilo body weight. The potassium inhibition thus established is allowed to continue for one minute before the second solution is injected. This is made up of 0.023 per cent $CaCl_2$ in 0.9 per cent NaCl. The purpose of this second injection is to remove the excess potassium from the coronaries and to substitute its antagonist, calcium. About ten cubic centimeters per kilo are injected; the injection is then stopped momentarily to introduce one cubic centimeter of 1:1,000 adrenalin chloride into the cannula and this is followed by a further fifteen cubic centimeters per kilo of the calcium solution.

While this procedure has led to some outstanding and remarkable resuscitations it is not considered to be fully perfected. The underlying principle is believed to be sound but further experimental modifications should be tested, particularly for application to animals which have been left in fibrillation for periods longer than five minutes and with the object of compensating for or avoiding the hydremic plethora which results.

This technical procedure was fully developed before attention was directed to a contribution by Crile and Dolley. In 1906 these authors published a paper on the resuscitation of dogs killed by anesthetics and asphyxia. Having tried 1, cardiac massage; 2, cardiac massage with artificial respiration, and 3, cardiac massage and artificial respiration with intravenous infusion of saline containing adrenalin, with limited success, they resorted to the infusion of Ringer into the central end of the carotid purposing to produce a coronary pressure of 30 to 40 mm. Hg, a pressure which Sollmann had reported as essential for normal cardiac activity. To the Ringer so used were added adrenalin and hirudin. This measure was accompanied by artificial respiration and rhythmic thoracic compression.

Crile and Dolley employed their procedure in dogs whose hearts had ceased to beat, as judged by the absence of peripheral pulse and heart sounds, following excess of chloroform and ether and asphyxiation. They report that dogs which showed no signs of cardiac activity for five minutes were uniformly and readily resuscitated; after ten minutes consciousness was rarely restored, while after twenty-three minutes in adult dogs and thirty-five minutes in puppies complete return of the circulation was not accomplished.

Although the experimental heart failure induced in these experiments is wholly different from ventricular fibrillation and although the significance of their criteria of death may be questioned, since many laboratory workers have revived animals some time after cardiac collapse from overdoses of anesthetic by artificial respiration alone, it is, nevertheless, of interest that the method which they found of greatest value is very similar to the method successfully employed in this research.

Since the condition to be overcome in the two investigations is wholly different it is not surprising that the basic arguments on which treatment is based should be different. As has been pointed out for the isolated heart Ringer solution is wholly inefficacious in overcoming ventricular fibrillation. A transitory excess of potassium is required. This accounts for the failure of Stanton and Krida (24) to resuscitate dogs, in which ventricular fibrillation was established, with the procedure of Crile and Dolley.

No particular effort was made to standardize the efficacy of the above described procedure in the sense of using the exact technique on a number of animals but its worth may be indicated by the results obtained in twenty-one consecutive experiments in which the technique was subjected to minor modifications such as the use and non-use of heparin and adrenalin, the amount of the two solutions injected per kilo, the strength of the solutions employed and the method of establishing fibrillation. In the first six and the last seven of these consecutive experiments the thorax was open and a normally beating heart was obtained in every case. In the eight intermediate experiments the thorax was intact In this group there was one conspicuous failure and one doubtful result, with six perfect recoveries. In the doubtful experiment (Jan. 6, 1928) the blood pressure record, after the precipitate fall incident to fibrillation, rose coincidently with the injections only to fall to the former low level and did not show arterial pulsations. Some thirty minutes later the abdomen was incised and the heart was palpated through the diaphragm. A slow, asphyxial ventricular beat was recognized. Neither adrenalin nor heparin was used in this experiment and the heart was not examined post mortem but in the light of later experience it is suspected that the blood had clotted in the left ventricle.

If this questionable result be excluded we have a series of twenty consecutive experiments with nineteen recoveries and one failure which may be taken to demonstrate the theoretical value of the procedure.

It is of passing interest to comment on the complete absence of failures in the chest-open experiments in which it is possible to observe the response of the heart because, as will be pointed out later, some simple criterion is needed to determine with accuracy if the potassium has produced complete ventricular quiescence in the intact animal. With the heart exposed it is quite simple to establish this fact but without opportunity for direct observation the full efficacy of the potassium treatment must be assumed.

It is appropriate here to consider the value of heparin and of adrenalin. In a considerable number of failures to resuscitate the heart the blood was found to have coagulated particularly in the left ventricle. That this was not a post-mortem phenomenon was indicated by an immediate examination. It appears, consequently, that the entrance of the foreign solutions is a contributing factor. This was especially evident when strong potassium solutions were introduced by ventricular puncture. Whether such coagu-

lation takes place in the coronary vessels is not known, nor is it known if the passage of an electric current is a predisposing factor to coagulation. The latter would be worth investigating since the coagula were by no means always limited to the left ventricle. They were frequently found in all four chambers of the heart. At any rate after the use of heparin with the potassiun solution was begun no further difficulty on this score was experienced.

The use of adrenalin to stimulate the heart was likewise not invariably essential. Whatever may be the mode by which adrenalin exerts its stimulant action it does not seem conspicuously to conduce to fibrillation. Repeated powerful doses introduced into the coronary circulation of the isolated heart increase the force and rate of beat but do not set up fibrillation. It is, of course, possible that the heightened activity thus produced might pass into fibrillation if the substance was applied over a longer period, as is the case with strong calcium chloride, but at present we have no definite evidence on this point. In a few experiments it might have been inferred that the use of adrenalin induced a return of fibrillation. But, on the other hand, and particularly when the heart was left in fibrillation for five minutes or more, the exhausted organ was obviously benefited by it and it was inferred that its vaso-constrictor action was of significant advantage in the recovery from the protracted period of circulatory failure. Consequently, while it is not at present possible to give conclusive evidence of the value of adrenalin as an aid in the resuscitation from fibrillation a considerable experience with its use in this condition leaves the impression that it is of real worth.

In two experiments (Feb. 23, Nov. 21, 1928), ephedrine sulphate and adrenalin chloride were compared as to their value in the resuscitation procedure. While the results are not considered conclusive the impression remains that adrenalin is decidedly superior.

Especial interest attaches to one of the experiments with the thorax intact (Jan. 16, 1928) because the remedial solutions were injected into the *femoral* artery. Only one experiment of this character was performed but it is of importance as indicating that another arterial route than the carotid may be utilized. Thus should the method ever be applied to resuscitation in man the brachial artery is appreciably more accessible than is the carotid. The protocol and part of the blood pressure record of this animal are given below.

The time factor in resuscitation. The favorable results thus far reported have not been related to the time in which the heart was left in fibrillation before remedial measures were instituted. Actually in four of these animals the heart was allowed to fibrillate for three minutes; in one, two minutes; in two, one and one-half minutes and in one, one minute. The

remainder (twelve) were left in fibrillation only long enough to make certain that the fibrillation was permanent.

As the period of fibrillation is increased the probability of a successful recovery decreases, as is shown in table 1.

It should be emphasized, as was pointed out above, that the results presented in this table are from experiments in which the technique was in process of development, which accounts for some of the failures. But as regards the time factor this is rather in favor of the experiments in which the fibrillation was prolonged since most of the efforts at resuscitation after five minutes or more were carried out late in the investigation.

It should be further pointed out that the resuscitation of the heart as thus far reported is not equivalent to permanent recovery, as will be seen when the protocols are examined. Most of the experiments on which the present paper is based were not concerned with the ultimate outcome of

TABLE 1

To show that the longer a heart fibrillates the more difficult and uncertain is resuscitation

LEFT IN FIBRILLATION	NUMBER OF EXPERIMENTS	FAILURES	DOUBTFUL	SUCCESSES
3 minutes or less	34	4	2	28
4 minutes	2			2
5 minutes	30	19	1	10
7 minutes	5	3	1	1
8 minutes or more	3	2		1

the procedure and the animals were sacrificed after the response of the heart was determined.

The greater difficulty of reviving a heart after a prolonged period of fibrillation raises the interesting question as to whether the fibrillation becomes so firmly fixed that the strength or amount of potassium chloride used was insufficient to induce adequate potassium inhibition. An answer to this question can probably be had when electrocardiographic records of the heart are taken during the procedure. There is no difficulty in reviving the asphyxiated heart with artificial perfusion and the isolated heart has been left in fibrillation for two hours, the perfusion being continuous, and recovered as readily with a little strong potassium chloride as is the case with a similar heart which has fibrillated but a short time. The question is: does fibrillation plus asphyxiation alter the problem? It is quite conceivable that a stronger potassium solution would be advantageous for hearts which have been in fibrillation for a long time.

In those intact animals which were subjected to prolonged fibrillation, i.e., more than three minutes, resort was had to intratracheal insufflation. This was done because, as will be seen in table 2 and in the protocols,

spontaneous respiration is resumed an appreciable time after the heart begins to beat and it was deemed advisable that the circulating blood should be aerated to lessen the asphyxial injury to the medullary centers.

Table 2 brings together the experiments bearing upon the time in which a heart may be left in fibrillation with prospects of recovery. The figures accord with those of table 1 and in addition indicate that complete recovery is not likely to result if fibrillation continues more than five minutes. It is possible, of course, that those animals which died after resuscitation was apparently established might have been saved with proper attention but

TABLE 2

Summary of experiments to show relation between recovery and duration of fibrillation

DATE	THORAX	HEART IN FIBRILLATION	HEART RECOVERED	RESPIRATION RECOVERED	EYE-LID REFLEX RECOVERED	COMMENT
1928		*minutes*	*minutes*	*minutes*	*minutes*	
March 2	Open	2	9	9		Sacrificed
March 8	Open	1½	8½	8½		Sacrificed
March 26	Closed	2				Complete recovery
March 30	Closed	5	7½	11		Sacrificed
April 2	Closed	18½	20			Transitory heart-beat and elevation of B. P. only
April 9	Closed	5	7¼			Died 39 minutes after fibrillation
1929						
January 23	Closed	5	7	10		Dead January 24
January 25	Closed	5	8½	12¼	30	Complete recovery
February 4	Closed	7	11⅓	$13\frac{2}{5}$	$28\frac{5}{6}$	Died 56 minutes after fibrillation
April 12	Closed	2	6	7		Complete recovery
April 16	Closed	5	14	17	35	Died about an hour later
May 3	Closed	5	8½	10½	21	Complete recovery

this carries the problem into another field since they did not die in ventricular fibrillation.

Attention is called to the successes of January 25 and May 3, 1929, in both of which the heart was left in fibrillation for five minutes, as indicating the period of asphyxia which the medullary centers will tolerate, ten and a half and twelve and a quarter minutes.

PROTOCOLS. *January 16, 1928*, male, 2.7 k. Morphia and ether. Both femorals exposed and cannulated; left, for blood pressure record, right, for injections. Tracheotomy for artificial respiration. Electrodes on either side of thorax.

Shocked with direct current, 110 volts, 1.0 ampere for 2 seconds. The heart was left in fibrillation about 2 minutes.

The numbers on the accompanying graphic record indicate: *1*. Application of

the electric current. *2.* Artificial respiration. *3.* Injection of 75 cc. KCl 0.5 per cent in NaCl 0.5 per cent, saturated with oxygen and warmed to body temperature. *4.* Injection of 75 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature. *5.* Injection of 125 cc. of same plus 10 cc. adrenalin 1:100,000. *6.* Artificial respiration off.

This procedure was repeated a second time with a similar result. The animal was then sacrificed.

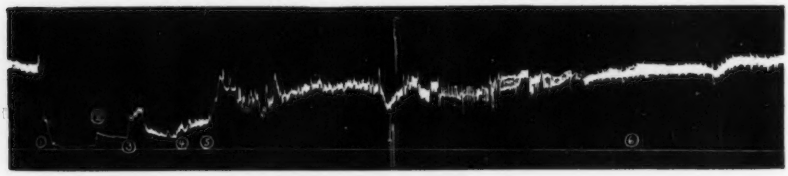

Fig. 1. Experiment of January 16, 1928

The graphic record of this experiment shows very slight pulsations following *2.* These are due to the artificial respiration. On the contrary the larger pulsations following *3*, during and after the potassium injection, represent ventricular action. The ability of the ventricles thus to resume orderly activity after or coincident with the injection of potassium was occasionally observed in other animals. It is presumably related to a nice balance of the amount of potassium entering the coronaries; sufficient, on the one hand, to stop the fibrillation, insufficient, on the other hand, to inhibit impulse formation. The vigor of the ventricular contractions was never adequate to restore normality, however, without the subsequent use of calcium.

January 24, 1928, female, 4 k. Morphia and ether. Right carotid and right femoral exposed and cannulated. Blood pressure record from femoral. Electrodes on either side of thorax.

Shocked with direct current, 110 volts, 0.3 ampere, for 5 seconds. The heart was left in fibrillation about 1 minute.

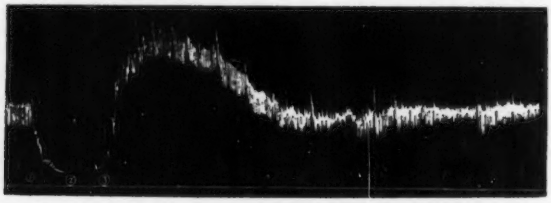

Fig. 2. Experiment of January 24, 1928

The numbers on the accompanying graphic record indicate: *1.* Application of the electric current. *2.* Injection of 35 cc. KCl 0.5 per cent in NaCl 0.5 per cent, saturated with oyxgen and warmed to body temperature. *3.* Injection of 50 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature, plus 5 cc. adrenalin 1:25,000.

After the establishment of normal conditions the cannulae were removed, the

wounds closed and the animal returned to the cage. The next day the dog was in excellent condition and was used for another experiment.

March 26, 1928, female, 3.6 k. Morphia and ether. Right carotid and right femoral exposed and cannulated. Blood pressure record from femoral. Electrodes on either side of thorax.

Shocked with direct current, 113 volts, 0.8 ampere, for five seconds. Heart left in fibrillation about three minutes.

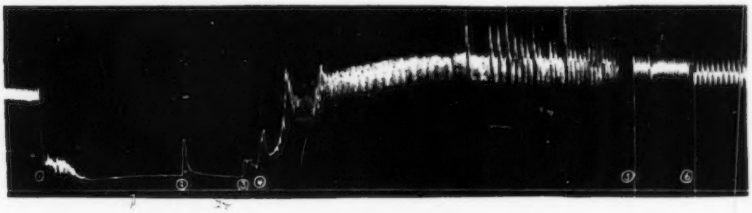

Fig. 3. Experiment of March 26, 1928

The numbers on the accompanying graphic record indicate: *1.* Application of the electric current. *2.* Injection of 25 cc. KCl 0.5 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature. *3.* Injection of 30 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature. *4.* Injection of 75 cc. of same plus 1 cc. adrenalin 1:1000. *5.* Drum stopped 10 minutes. *6.* Drum stopped 10 minutes.

Fig. 4. Experiment of March 26, 1928. Photographed March 28th

The cannulae were removed, the wounds closed and the animal returned to cage. On March 28th the accompanying photograph was taken. This dog was used in the subsequent experiment of March 30th.

March 30, 1928 (animal used in experiment of March 28th). Morphia and ether. Right carotid and right femoral exposed and cannulated. Blood pressure record from femoral. Electrodes on either side of thorax.

Shocked with direct current, 111 volts, 0.8 ampere, for 5 seconds. Instituted intratracheal insufflation. Heart left in fibrillation 5 minutes.

The numbers on the accompanying graphic record indicate: *1.* Application of the electric current. *2.* Stopped drum. Rhythmic abdominal compression instituted and continued until potassium injection. *3.* Injection of 28 cc. KCl 0.5 per cent in

NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature. *4.* Injection of 35 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature. *5.* Injection of 50 cc. of same plus 1 cc. adrenalin 1:1000. *6.* Injection of 50 cc. of same plus 1 cc. adrenalin 1:1000. 7. Removed insufflation tube. 8. Stopped drum for 15 minutes.

In this animal the heart-beats began 7 minutes, 30 seconds after fibrillation was established.

Sacrificed.

April 2, 1928, female, 2 k. Morphia and ether. Right carotid exposed and cannulated for injection, right femoral for blood pressure record. Electrodes on either side of thorax.

Intratracheal insufflation begun and ether administered to complete abolition of eye-lid reflex. Shocked with direct current, 114 volts, 0.6 ampere, for 5 seconds.

Waited 8 minutes, 30 seconds, during which time rhythmic abdominal compression was applied, and injected 23 cc. KCl 0.5 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature.

Fig. 5. Experiment of March 30, 1928

Waited 1 minute and injected, in divided doses, 160 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent plus 3.5 cc. adrenalin 1:1000. The heart did not respond.

Eighteen minutes after fibrillation injected 30 cc. of potassium, followed, after 1 minute, by 60 cc. of calcium solution with 2 cc. adrenalin 1:1000.

After twenty minutes the heart was beating normally and the blood pressure had risen to about 60 mm. Hg. The heart beats and blood pressure gradually faded away and after twenty-six minutes 65 cc. calcium solution plus 2 cc. adrenalin were injected with transitory benefit. The animal was dead thirty minutes after fibrillation.

January 23, 1929, female, 5 k. Morphia and ether. Right carotid exposed and cannulated. Electrodes on either side of thorax. Ether to abolition of respiration. With first natural breath shocked with direct current, 110 volts, 0.6 ampere for 2 seconds. No pulse. Intratracheal insufflation begun.

Waited 5 minutes and injected 60 cc. KCl 0.5 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature, containing 4.5 mgm. heparin, under 150 mm. Hg pressure.

Waited 1 minute and injected 50 cc. $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent saturated with oxygen and warmed to body temperature and 2 cc. adrenalin 1:2000 three times under same pressure, coupled with manual compression of abdomen and thorax.

Seven minutes after fibrillation heart beating strongly; first natural respiration 3 minutes later.

Cannula removed and wound closed. Animal returned to cage.

Found dead next moring; had not moved. Necropsy—lungs normal; heart small and hard, blotchy and dark; left ventricle empty; right ventricle filled with diffuse clot; abdomen distended; intestines filled with gas; large volume of bloody fluid with

clots in abdominal cavity; bladder small and contracted; spleen and liver of normal size.

January 25, 1929, adult female, +5 k. Morphia and ether. Right carotid exposed and cannulated. Electrodes on either side of thorax.

Shocked with direct current, 110 volts, 0.5 ampere for 2 seconds. Intratracheal insufflation begun.

Waited 5 minutes and injected 55 cc. KCl 0.5 per cent in NaCl 0.9 per cent saturated with oxygen and warmed to body temperature.

Waited 1 minute, 20 seconds and injected 50 cc. $CaCl_2$ 0.119 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature, and 2 cc. adrenalin three times under 150 mm. Hg pressure.

First heat beat 8 minutes, 30 seconds after fibrillation.

First spontaneous respiration 12 minutes, 15 seconds after fibrillation.

Closed wound, removed insufflation tube and returned to cage 45 minutes after fibrillation.

This dog made a good recovery and when sacrificed on February 27th had gained approximately a kilo in weight. There was, however, a definite impairment in vision such that she did not avoid objects in her path. The presence or absence of visual disturbance was not noted before the experiment. Specimens of the tissues were preserved for microscopic study. The accompanying photograph was taken February 18th.

Fig. 6. Experiment of January 25, 1929. Photographed February 18th

February 4, 1929, young male, 2.5 k. Morphia and ether. Right carotid exposed and cannulated. Electrodes on either side of thorax.

Shocked with direct current, 110 volts, 0.7 ampere, for 2 seconds. Intratracheal insufflation begun.

Waited 7 minutes and injected 29 cc. KCl 0.5 per cent NaCl, saturated with oxygen and warmed to body temperature, containing 12 mgm. heparin under 150 mm. Hg pressure.

Waited 1 minute, 15 seconds and injected 80 cc. $CaCl_2$ 0.2 per cent in NaCl 0.9 per cent, saturated with oxygen and warmed to body temperature, with 2 cc. adrenalin 1:2000 at 150 mm. Hg pressure.

At 10 minutes, 30 seconds injected 2 cc. adrenalin 1:2000 in 25 cc. of calcium solution.

At 11 minutes, 20 seconds, first heart beat.

At 13 minutes, 8 seconds, first gasp.

At 16 minutes, stopped insufflation.

At 19 minutes, 30 seconds, pulse weak; injected 1 cc. adrenalin 1:2000 in 15 cc. of calcium solution.

At 28 minutes, 50 seconds, eye-lid reflex obtained.

At 37 minutes, stopped insufflation.

At 51 minutes, finished closing wound.

At 56 minutes, 20 seconds, animal unexpectedly died.

Necropsy—abdominal viscera dry and pale—no fluid; lungs good; heart not engorged; no clots; ventricular blood watery but of good color.

April 12, 1929, female puppy, 4 5 k. Morphia and ether. Right carotid exposed and cannulated. Electrodes on either side of thorax.

Shocked with direct current, 110 volts, 0.7 ampere for 2 seconds.

Waited 2 minutes and injected 51 cc. KCl 0.5 per cent in NaCl 0.5 per cent, saturated with oxygen and warmed to body temperature, at 150 mm. Hg pressure.

Waited 1 minute and injected 50 cc. $CaCl_2$ 0.023 per cent in 0.9 per cent NaCl, saturated with oxygen and warmed to body temperature.

Waited 30 seconds and injected 50 cc. more of calcium solution with 2 cc. adrenalin 1:2000.

Waited 1 minute, 45 seconds, and injected 25 cc. more of calcium solution with 2 cc. adrenalin 1:2000.

Six minutes after fibrillation heart beating strongly.

Seven minutes after fibrillation breathing naturally.

Closed wound and returned animal to cage.

April 13, weak but making good progress; no visual disturbance.

April 14, animal in good condition but had torn open and soiled wound badly; sacrificed.

May 3, 1929, immature female, 5.25 k. Morphia and ether. Right carotid exposed and cannulated. Electrodes on either side of thorax.

Shocked with direct current, 110 volts, 0.6 ampere for two seconds. Intratracheal insufflation begun.

At 5 minutes after fibrillation injected 61 cc. KCl 0.5 per cent in NaCl 0.5 per cent, saturated with oxygen and warmed to body temperature, containing 15 mgm. heparin, at 150 mm. Hg pressure.

At 6 minutes injected 60 cc. $CaCl_2$ 0.023 per cent in 0.9 per cent NaCl, saturated with oxygen and warmed to body temperature.

At 7 minutes, injected 60 cc. more of same with 2 cc. adrenalin 1:2000.

At 8 minutes, 20 seconds, heart beating.

At 10 minutes, 30 seconds, natural breathing resumed.

At 21 minutes, eye-lid reflex present; insufflation tube removed.

At 39 minutes, closed wound and returned to cage.

May 4, weak but in excellent condition; drank milk, voided and defecated; no visual disturbance.

May 6, well and playful; appetite good.

May 7, weight 5.7 k.; motion picture, shown at International Physiological Congress in Boston, taken.

This dog was sterilized on May 8th and became a pet of the writer's children.

The foregoing presentation has emphasized the efficacy in the resuscitation from ventricular fibrillation of the use of potassium and calcium, with adrenalin, when introduced through the cannulated carotid. Certain other theoretically simpler or more efficacious methods for the introduction of these substances into the coronaries, however, deserve brief consideration and their comparative merits may now be presented.

Comparison of the ventricular and carotid routes. It has been shown above that ventricular absorption is of little or no promise from a practical standpoint; nevertheless it seemed advisable to compare this form of administration with that of the carotid route. A report of the decisive experiment of March 1, 1928, will suffice to make the point perfectly clear. In this experiment the animal was anesthetized with morphia, veronal and ether. The thorax was opened under artificial respiration, the pericardium was

incised and the right carotid cannulated. The solutions to be used, KCl 0.5 per cent in NaCl 0.9 per cent and $CaCl_2$ 0.023 per cent in NaCl 0.9 per cent, were saturated with oxygen and placed under a pressure of 150 mm. Hg. The ventricles were fibrillated by direct application of a tetanic stimulus.

After fibrillation, 59 cc. of the potassium solution were introduced into the left ventricle through a large hypodermic needle. The fibrillation stopped and the heart rested for 30 seconds when an unrecorded amount of the calcium solution plus 1 cc. of adrenalin 1:1000 was introduced by the same route; fibrillation returned.

The heart remained in fibrillation at least 5 minutes, when 52 cc. of the potassium solution were injected via the carotid. The fibrillation stopped and the heart rested for 30 seconds. This was followed by an unrecorded amount of the calcium solution plus 3 cc. adrenalin 1:1000. A normal sequential beat was established.

Ten minutes elapsed and the heart was again fibrillated. The fibrillation was stopped by ventricular injection of 67 cc. of the potassium solution and the heart rested for 1 minute. This was followed by the same route with an unrecorded amount of calcium solution plus 2 cc. adrenalin 1:1000. Fibrillation returned.

Again 35 cc. of the potassium solution by way of the carotid stopped the fibrillation and brought the heart to rest. An unrecorded amount of the calcium solution plus 1 cc. of adrenalin 1:1000 introduced through the carotid once more developed a normal beat.

These results show that 1, while potassium introduced via ventricular puncture may stop fibrillation, it requires a greater amount than is needed if the carotid route is followed; 2, washing out the potassium via the ventricular route is followed by fibrillation while if it is washed out by the carotid route a normal beat is established, and 3, the amount of adrenalin used is not a contributing factor in the results obtained.

The conclusions drawn from this experiment, namely, that the carotid is superior to the ventricular route, are supported, although less convincingly, in five other experiments.

Introductions of solutions through a carotid sound. One of the disadvantages of the method of introducing the solutions via the carotid was the large amount of fluid required. The intrathoracic and abdominal hemorrhages which were the probable cause of death in some cases were accounted for by the resultant plethora accentuated by manual artificial respiration. Although the volume of fluid used in the later experiments was successfully tolerated it seemed desirable to experiment with a sound passed down the carotid to the aortic arch through which the solutions, under the carrying pressure of 150 mm. Hg, might be less dissipated in the vascular bed in approaching the coronary orifices.

Four experiments of this character were performed, two with the thorax opened and two with it intact. With the chest open and the heart visible it was possible to place the sound nicely and the outcome was promising as seen in the following protocol:

February 20, 1929, female, 5 k. Morphia and ether. Tracheotomy and open thorax. Sound, filled with NaCl 0.9 per cent, passed into carotid as far as the second intercostal space. Solutions saturated with oxygen and warmed to body temperature.

Tetanic stimulus for 5 seconds, fibrillated heart.

Waited 1 minute and injected 12 cc. KCl 0.5 per cent in NaCl 0.5 per cent. Heart rested. Waited 1 minute and injected 40 cc. $CaCl_2$ 0.3 per cent in NaCl 0.7 per cent. Waited 20 seconds and injected 1 cc. adrenalin with 15 cc. of calcium solution.

The heart was beating rapidly 2 minutes, 50 seconds after fibrillation, but shortly returned to a normal rate.

The procedure was repeated but with only fair success because the blood had clotted in both ventricles.

In the two experiments with the thorax intact coagulation was obviated by the use of heparin but in both the ultimate outcome was unfavorable. Thus in the experiment of February 25, 1929, having failed to recover the heart in one attempt the thorax was opened, without disturbing the position of the sound, and two injections of 20 cc. and 25 cc. of the potassium solution were made. Neither of them brought the heart to rest.

The difficulty of introducing the sound, the danger of injury to the intima of the vessel and the unfavorable results obtained in the two animals with the thorax intact indicated that use of an arterial sound for the injections was disadvantageous and the method was not pursued further.

Use of hollow needle for carotid injections. For practical reasons this method is deserving of consideration even though it failed in five experiments. The technique of introducing a needle into the lumen of the collapsed carotid is quite difficult and a needle suitable for such introduction has too small a bore to permit the free entrance of fluid for the purposes in hand. It may be doubted if a needle, under the most favorable circumstances and in the most skilled hands, would be superior to a suitably constructed cannula in an emergency.

SUMMARY. It is realized that the presentation in the foregoing pages is somewhat involved and it is therefore desirable to offer a general survey of the findings which have been reported.

Ventricular fibrillation is believed to be the most common cause of death in electric accidents. In the dog, as is assumed to be the case in man, ventricular fibrillation, when once established, is all but invariably permanent with resultant death since the fibrillating ventricles are incompetent to maintain the circulation.

Potassium chloride is the only agent of demonstrated efficacy in overcoming ventricular fibrillation. It acts by producing a state of inhibition.

This inhibition, in turn, can be overcome by removing the excess potassium from the coronary vascular bed.

With these facts in mind experimentation led to a procedure suitable for the resuscitation of dogs in which ventricular fibrillation had been induced by electric shock. This procedure calls for the central carotid injection of the remedial solutions, saturated with oxygen and warmed to body temperature, at a pressure of 150 mm. Hg. The arterial pressure being practically at zero, the solutions thus injected readily find their way into the coronary arteries. The first solution (KCl 0.5 per cent, NaCl 0.9 per cent plus heparin 0.25 mgm. per cc.), about 13 cc. per kilo, establishes potassium inhibition and the heart is allowed to rest for one minute. The second solution ($CaCl_2$ 0.023 per cent, NaCl 0.9 per cent), about 25 cc. per kilo, washes the excess of potassium out of the coronary bed and substitutes a relative excess of calcium chloride the effect of which is to stimulate a normal cardiac rhythm. This effect is enhanced by the coincident injection of 1 cc. of 1:1000 adrenalin chloride.

Such an approach to the problem of resuscitation from ventricular fibrillation has not theretofore been suggested and it has resulted in a number of spectacular recoveries. The method should, however, be subjected to further critical study before considering its application to man because there are obvious indications that it may be improved, particularly for use in cases of long-lasting fibrillation.

CONCLUSIONS

1. Methods heretofore suggested for the recovery of the heart in electric shock have not been found efficacious.

2. A new method, based on physiological principles, is described and the results from its use are reported.

3. This method, although complicated and difficult of practical application, seems, as judged from the results obtained, to promise a real advance in the control of ventricular fibrillation.

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FURTHER OBSERVATIONS ON THE ACTION OF "CHOLECYSTOKININ"

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In this paper we will report a number of observations that have been made on various points that have occurred to us during the course of our study of the action of "cholecystokinin" on the gall bladder.

The visual observation of gall-bladder contractions: Ivy and Oldberg (1928) reported in their article that they observed under the fluoroscope the gall bladder visualized with lipoidol to undergo a change in contour following injections of "cholecystokinin." These changes were most evident in the half adjacent to the neck of the gall bladder. Further, changes in contour and evacuation were recorded on x-ray plates by them.

As is well known, the gall bladder in most dogs lies rather far up under the chest wall and can be seen only with difficulty. We have constantly been on the alert for dogs whose gall bladders could be easily seen through an abdominal incision and which were relatively free from attachments to the liver, for the purpose of ascertaining whether or not we could see the gall bladder contract when "cholecystokinin" was injected. Up to the present time we have observed two such gall bladders, the contraction of which we shall describe.

In the first case, on opening the abdomen of a "flat chested" bull dog, the gall bladder was just beneath the upper portion of the incision and two-thirds of it "hung free," or was not attached to the liver. The cystic duct was clamped and the dome of the gall bladder was cannulated to record changes in pressure. On the intravenous injection of two doses of a "cholecystokinin" preparation the intra-gall-bladder pressure started to rise. Coincidently with this rise, a circular band of contraction, about 2 cm. across, appeared in the mid-portion of the gall bladder together with some longitudinal shortening. As the intra-gall-bladder pressure began to decline and coincident with the appearance of a tonus rhythm, a circular band of contraction about 3 mm. in diameter was seen to appear and disappear synchronously with each tonus rhythm. (Observed by Lueth, Kloster, Ivy, Dr. A. I. Kendall and others.) Figure 1 shows a tracing from this dog following an injection which caused a visible contraction without tonus rhythm.

In the second case the gall bladder was also easily seen through the abdominal incision, but was not so nearly free from the liver as was true of the first case. The cystic duct was not clamped, neither was the gall bladder cannulated. On the intravenous injection of three doses of a "cholecystokinin" preparation, the gall bladder was observed to shorten longitudinally, its dome being erected, or drawn forward, and to decrease in circumference and volume. Two more injections decreased the size of the gall bladder approximately seventy-five per cent within a period of twenty minutes. (Observed by Lueth, Drewyer, Ivy, Dr. H. Schmitz and others.)

The two-thirds of the gall bladder adjacent to the cystic duct is the most contractile portion, according to our observations. The type of contraction observed by us resembles that described by Whitaker (1926) for the cat, by Higgins and Mann (1926) for the guinea pig, and by Levine (1927) for man. The contraction of the gall bladder of the dog, or the contraction as it was described above, was as evident as contractions of the gall bladder of the guinea pig.

Effect of "cholecystokinin" on liver volume: Since the gall bladder is in contact with the liver and not infrequently two-thirds surrounded by the liver, it is possible that a change in liver volume might cause changes in intra-gall-bladder pressure. We did not think that the increases in intra-gall-bladder pressure observed by us on the injection of vaso-dilatin free "cholecystokinin" were due to increased liver volume because it was difficult to conceive how liver volume could be materially influenced without a fall in general blood pressure. However, Griffith and Emery (1929) report that small doses of epinephrin via the femoral vein may produce a dilatation of the liver with or without a fall in the general blood pressure.

In order to ascertain definitely whether or not a change in liver volume was responsible for the change in intra-gall-bladder pressure we were observing, we adopted a method used by Bainbridge and Dale (1905). A rubber balloon (condom) the size of the gall bladder was inserted between the two large lobes of the liver so that it was completely covered by these lobes and held in place by a low tension stitch through the edges of each lobe. The balloon was partially filled with air (from 5 to 10 cc.) and connected to a "Becker" tambour or the kind that was being used for recording the gall-bladder changes, the lever arms of the two tambours being identical.

In every experiment it was found that the "cholecystokinin," which contained no vaso-dilatin, caused no change in liver volume, but a rise in intra-gall-bladder pressure occurred. If vaso-dilatin was added to the preparation, or if a preparation was used which contained vaso-dilatin, there occurred an increase in liver volume which coincided with the fall in blood pressure, returning to normal in a few minutes; but the gall-bladder pressure did not return to normal until fifteen or twenty minutes later (figs. 2 and 3).

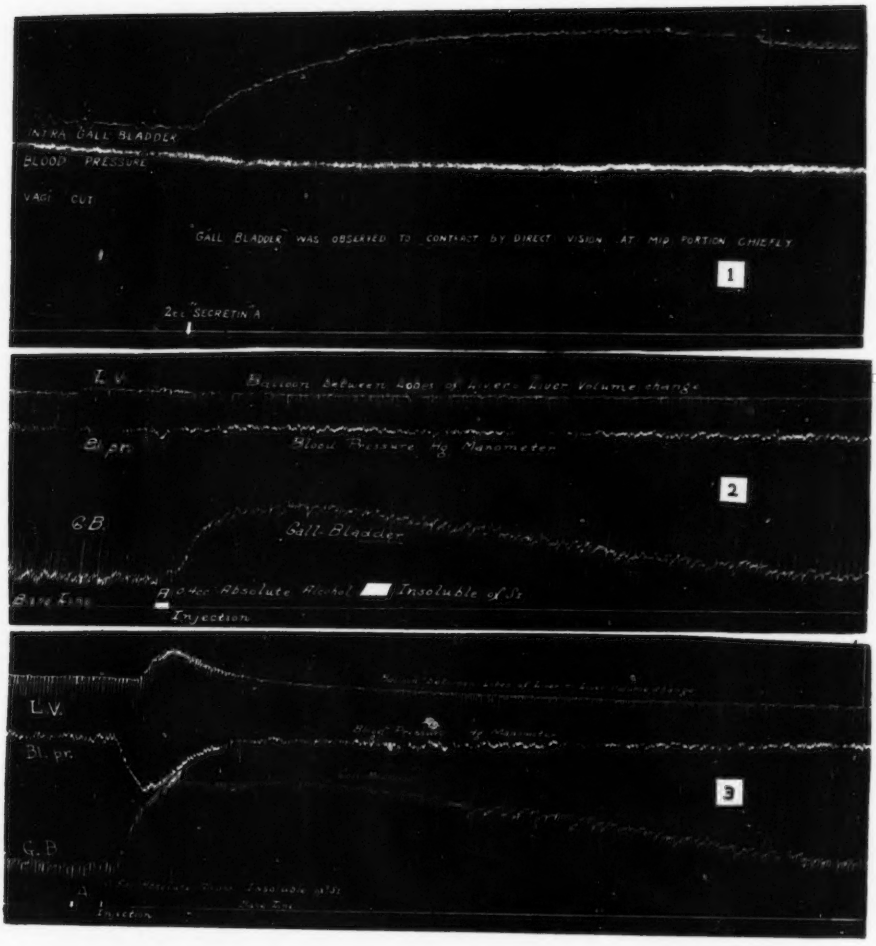

Fig. 1 shows a rise in intra-gall-bladder pressure that occurred in an experiment in which the contraction of the gall bladder was observed by direct vision. Following this particular injection a "tonus rhythm" did not occur, which was observed in the preceding injections and which is seen on this tracing prior to the injection of preparation "secretin" A.

Fig. 2 shows a record of liver volume, *L V.*, from the artificial gall bladder between the lobes of the liver (note absence of tonus rhythm on this tracing), of the blood pressure, and of the intra-gall-bladder pressure. Note that on the injection of "cholecystokinin" which contained no "vaso-dilatin," the gall bladder contracted without a change in liver volume.

Fig. 3 shows a similar record to that of figure 2 but in which vaso-dilatin was present in the "cholecystokinin" preparation. Note that the fall of blood pressure and rise in liver volume occur concomitantly, but the rise in gall-bladder pressure persists for more than 15 minutes.

This result is not surprising when viewed in the light of the results of Ivy and Oldberg (1928) on the effect of histamine on the gall-bladder pressure. They observed that when histamine was injected, the gall-bladder pressure was increased but returned to normal as the blood pressure returned to normal, whereas "cholecystokinin" caused a rise in gall-bladder pressure which persisted for from ten to sixty minutes.

Effect of "cholecystokinin" on the motility of the stomach and intestine: In considering the extrinsic factors that might lead to a rise in intra-gall-bladder pressure, a factor that must be considered is the movement of the stomach or intestine, or contiguous contractile viscera. The possibility that the movement of the stomach or intestines may cause the change in the gall bladder is quite remote when one studies the type of contraction that occurs. However, in order to obtain definite evidence on this possibility and to ascertain whether or not "cholecystokinin" is more or less specific, we have studied the effect of solutions containing the active principle on the movements of the stomach and duodenum.

This study was made on anesthetized (barbital) and unanesthetized dogs. On the anesthetized dogs the gall bladder was prepared for recording intra-gall-bladder pressure, the carotid was connected to a manometer for recording blood pressure, and the stomach was incised ($\frac{1}{2}$ inch) in the fundus and a balloon (condom) placed in the first six inches of the duodenum and another balloon in the pyloric antrum. The experiments on the unanesthetized dogs were performed on dogs with a gastrostomy and a Thiry fistula of the jejunum so that gastric and intestinal movements could be recorded simultaneously.

Four experiments were performed on the anesthetized dogs, a number of injections of "cholecystokinin" being made in each experiment. In every experiment and test, the gall bladder contracted without any change in the motility of the stomach and intestine.

Three dogs with a gastrostomy and a Thiry fistula of the intestine were used and a large number of injections were made. The dogs were trained so that the insertion of the hypodermic needle into the vein caused no inhibition of motility. It was found that the "cholecystokinin" had no effect on the motility of the empty stomach during hunger periods and rest periods. The results on the intestine were less definite and very variable. Sometimes the motility of the jejunum was increased, sometimes decreased, and at other times was unaffected. This variability in effect was not due to the state of tonus of the jejunum. (These experiments on unanesthetized animals were done by Mr. J. P. Burgess.)

We had expected to find that "cholecystokinin" would cause an increase in intestinal motility because we frequently observe contractions of the intestine in barbitalized dogs following injections of "cholecystokinin"

in the course of our experiments on assaying various dilatin-free preparations.

We can state from the results of these observations that "cholecystokinin" has no effect on the motility of the stomach, whereas its effect on the intestine is so variable that it cannot be predicted.

The effect of morphine and atropine on the gall bladder: During the course of our experiments on the assay of various preparations, we have resorted to the use of morphine ($\frac{1}{4}$ grain hypodermically) in our lightly barbitalized dogs in order to avoid the use of ether. In approximately forty per cent of these dogs a marked tonus rhythm appeared shortly after the injection of the morphine, amounting to as much as five centimeters of bile pressure in one case.

We feel quite certain that this was due to the morphine and not to the withdrawal of ether, as some of the dogs in which we used the morphine had not received ether for twenty or thirty minutes previous to the morphine injection without the appearance of a tonus rhythm. The morphine was used to stop shivering and rigidity. The injection of "cholecystokinin" after the onset of the marked tonus rhythm caused the gall bladder to contract. Atropine sulphate ($\frac{1}{150}$ grain) had either no effect or a negligible one on the tonus rhythm.

It was shown by Ivy and Oldberg (1928) that atropine sulphate did not prevent the action of "cholecystokinin." This we have confirmed in more than ten experiments. Bainbridge and Dale (1905) found that atropine causes the gall bladder to relax.

Effect of ergotamine and magnesium sulphate on the gall bladder: Ergotamine tartrate (Sandoz) in from 0.2 mgm. to 1.5 mgm. doses given very slowly intravenously causes a slight contraction of the gall bladder, after which "cholecystokinin" causes a marked contraction. Bainbridge and Dale (1905) observed that chrysotoxin increases the tonus rhythm.

Two hundred milligrams of magnesium sulphate given intravenously to 15 kilo dogs does not prevent the action of "cholecystokinin" on the gall bladder, but this quantity definitely decreases the amount and duration of the contraction.

Effect of bile and bile salts on gall bladder: Ivy and Oldberg (1928) reported that bile and bile salts had no effect on the gall bladder. Because our purified preparations gave a positive Pettenkofer test we repeated the above work and confirmed it. Ten cubic centimeters of gall-bladder bile diluted ten times and slowly injected intravenously causes a slight relaxation of the gall bladder. One hundred milligrams of sodium glycocholate, if it has an effect, causes the gall bladder to relax. Bainbridge and Dale (1905) found that bile salt intravenously relaxed the gall bladder.

Contraction of the gall bladder after cervical transection: In two out of five

experiments in which the cord was sectioned at the third cervical level and the vagi cut, the animal being on artificial respiration, the injection of "cholecystokinin" caused the gall bladder to contract. This procedure, as is well known, causes a marked fall in blood pressure, which we believe accounts for the three failures, since it has been observed by us that deep ether anesthesia and low blood pressure abolishes or decreases the "cholecystokinin" effect.

This procedure was carried out in order to ascertain if the diaphragm and thoracic muscles played a part in the rise in the gall-bladder pressure being observed by us on injecting "cholecystokinin," which we had previously shown not to be likely by pneumograms made from thoracic movements. In this connection it should be pointed out that in all these experiments the abdomen is opened by a five or six inch incision, which is covered during the course of the experiment with a piece of cotton.

Spontaneous contraction of the gall bladder: Doyen (1893), Bainbridge and Dale (1905) and others have observed the gall bladder to undergo a genuine rhythmic contraction and relaxation, which we have called the "tonus rhythm" of the gall bladder. On several occasions in more than three hundred experiments on dogs we have seen a spontaneous contraction of the gall bladder to occur for which we could not account. In one experiment following the injection of a preparation of "cholecystokinin" which contained some vaso-dilatin, a spontaneous contraction occurred during the period of relaxation following the contraction caused by the injection.

On the relaxation of the gall bladder after "cholecystokinin": Ivy and Oldberg (1928) reported that following an injection of "cholecystokinin" the gall-bladder pressure would return to normal in from 10 to 60 minutes Since the writing of that paper, we have observed a number of dogs in which the gall bladder would not relax completely after the injection of a single dose of "cholecystokinin," but would remain contracted for more than two hours. In such animals we have given several doses of "cholecystokinin" with the result that a steplike rise in intra-gall-bladder pressure occurred so that the final rise in pressure amounted to from 15 to 20 cm. of bile pressure. Still the gall bladder would not relax appreciably after two or three hours. The administration of ether will cause such a gall bladder to relax but not completely.

Response for different animals: We have found that the gall-bladder of the cat and dog will contract on the injection of "cholecystokinin." We have not been able to record a contraction of the gall bladder of the rabbit and doubt that the rabbit's gall bladder will contract appreciably under the influence of "cholecystokinin." We have not yet injected it into man. Dr. G. E. Burget to whom we sent a preparation of "cholecystokinin" reported that it caused contraction of the gall bladder, bile duct, ampulla, and increased the motility of the duodenum in the guinea pig.

SUMMARY

1. The gall bladder has been observed to contract under direct vision following the injection of "cholecystokinin."

2. "Cholecystokinin" does not have any effect on liver volume. If vasodilatin is present in the preparation of "cholecystokinin," the change in intra-gall-bladder pressure lasts much longer than the change in liver volume due to the dilatin.

3. "Cholecystokinin" has no detectable effect on gastric motility. Its effect on intestinal motility is variable.

4. Morphine hypodermically in some animals causes an exaggerated "tonus rhythm" of the gall bladder, which is not materially affected by atropine. Atropine, however, causes some relaxation of the gall bladder.

5. Bile and bile salts administered intravenously cause a relaxation of the gall bladder in effective doses.

6. Ergotamine tartrate stimulates the gall bladder and has no effect on "cholecystokinin" action. Magnesium sulphate in 200 mgm. doses (30 lb. dog) diminishes the "cholecystokinin" action, but does not abolish it.

7. The diaphragm, thoracic and abdominal muscles play no part in the action of "cholecystokinin" on the gall bladder.

8. An occasional spontaneous contraction of the gall bladder occurs in some dogs which cannot be accounted for.

9. Dogs vary in their response to "cholecystokinin," the usual effect lasting for from 10 to 60 minutes. Occasionally the gall bladder will not relax, or return to normal for two or three hours after an injection

10. The gall bladder of the cat and dog are caused to contract on the intravenous administration of an adequate dose of "cholecystokinin." We have failed to record a contraction for the gall bladder of the rabbit.

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ON THE PREPARATION OF "CHOLECYSTOKININ"

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It has been demonstrated by Ivy and Oldberg (1928) that a purified extract of the upper intestinal mucosa contains a substance, which on intravenous injection, causes the gall bladder to contract and evacuate, and that the results of cross-circulation experiments yielded evidence which led them to the conclusion that a hormone mechanism is concerned in gall bladder evacuation.

In this paper we desire to report on the methods used to prepare purified preparations of intestinal extract which cause the gall bladder to contract.

METHOD OF ASSAY. The method for assaying the various preparations was that used by Ivy and Oldberg (1928), which consisted in clamping the cystic duct, cannulating the gall bladder through its fundus or dome, and connecting the cannula with a recording tambour. The carotid artery was connected with a recording blood pressure apparatus for a blood pressure tracing to detect the absence or presence of vaso-dilatin. Dogs were used throughout. Injections were made into the femoral vein.

METHODS OF PREPARATION. Since we have been working on this problem for two years, many methods have been used and many different preparations (over 3000) have been made. In this paper we will describe those methods that have most consistently yielded an active preparation.

We will first describe the method which has proven to be uniformly successful in yielding a product free from vaso-dilatin and causing the gall bladder to contract in from 3 to 6 mgm. (from dog's mucosa) and 5 to 10 mgm. (from hog's mucosa) doses.

The "A pH 1802 method": 1. The first six feet of the intestine are removed (within 20 minutes after death) from a freshly slaughtered animal (dog or hog). The intestine is tied at one end and filled with 100 cc. (dog), or 200 cc. (hog) of 0.4 per cent HCl in water. The other end is then tied. The intestine may be turned inside out and tied at both ends and immersed in the 0.4 per cent HCl solution with occasional stirring, which is the method we use when working with a large number of hog intestines. At the end of half an hour the acid solution is removed or poured from the intestines. This extraction procedure can be economically repeated

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three times. The acid extract is strained through gauze, a clear filtrate not being necessary at this point. 2. To the strained solution there are added from 25 to 30 grams per 100 cc. of sodium chloride (U. S. P.). The salt should be added to the solution as quickly as possible after removal from the intestines to prevent further formation of vaso-dilatin. The salt causes a precipitate to form (Weaver, Luckhart, Koch, 1926). It is best to allow from 1 to 12 hours for the precipitate to form. 3. The precipitate which we call "A," and which contains the active substance is collected in centrifuge tubes or on a filter paper. Most of the vaso-dilatin, but rarely all, especially if hog intestines are used, remains in solution in the filtrate. It is not advisable to wash this precipitate with 0.4 per cent HCl saturated with salt as some activity is lost. 4. The precipitate is suspended or dissolved in from $\frac{1}{2}$ to $\frac{3}{4}$ of the original volume of water. (The precipitate from the dog's intestine is frequently completely soluble, but rarely is this true if hog intestines are used.) 5. The reaction of the solution is now brought to a pH of 5.0−5.5 with NaOH where a flocculent precipitate forms. Care must be exercised at this point in order to obtain a clear filtrate, which is necessary. Allow to stand $\frac{1}{2}$ to 1 hour before filtering. This procedure removes much impurity. 6. Filter; the filtrate should be clear, if step 5 has been carefully done. The activity is in the filtrate. 7. To the filtrate, sufficient 20 per cent trichloracetic acid is added to make a concentration of 5 per cent trichloracetic acid. After adding the trichloracetic acid, the solution is agitated to insure thorough mixing and then it is allowed to stand without agitating from 1 to 4 hours. A flocculent precipitate forms. Sometimes it is necessary to add a little glacial acetic acid ($\frac{1}{2}$ cc. per liter) or 5 cc. of 5 per cent H_2SO_4 to obtain maximum flocculation. It is not advisable to let the precipitate remain too long in the trichloracetic acid solution. 8. The precipitate which contains the active substance is collected in centrifuge tubes or on filter paper in the form of a thick paste. Filter paper is employed when large quantities of fluid are being worked up, in which case the precipitate should not be dried to the extent that it turns brown. The filtrate should be clear, but sometimes it is uncontrollably cloudy. On standing overnight, this cloudy filtrate will clear up, but the resulting precipitate is inactive. 9. The trichloracetic precipitate is washed with absolute acetone (aldehyde free), using a sufficient quantity to insure more than a 95 per cent acetone solution, taking into account the water present in the pasty precipitate. 10. The acetone is decanted or filtered. 11. The precipitate is washed with anhydrous ether (C. P.), using about the same volume as that of the acetone used. 12. Decant, or filter the ether. 13. The residue, being very light, is dried carefully with a current of air or in a desiccator. This powder contains the active substance and has in our hands been uniformly vaso-dilatin free. We call it "A pH 1802."

This powder is usually entirely soluble in distilled water in a 1 per cent solution, 95 per cent phenol and in 70 per cent alcohol. The solution, however, is occasionally cloudy.

This procedure has never failed in our hands, being repeated many times, and has been adopted by us as a uniform procedure for preparing material for further purification.

The following is a typical experiment and yield: we took sufficient 0.4 per cent HCl solution and salt to the stock yards to work up 200 pieces of hog intestines. The 200 intestines were divided into three series: one series of 50, a second series of 50, and a third series of 100. The above procedure was followed through step 2 at the stock yards, and then through step 13 at the laboratory. The first series of 50 gave a yield of 1600 doses, active in 6.6 mgm. doses, and vaso-dilatin free. The second series of 50 gave a yield of 2000 doses, active in 4.6 mgm. doses and vaso-dilatin free. The third series of 100 gave a yield of 3400 doses, active in 6.5 mgm. doses and vaso-dilatin free.

In order to test our procedure further, directions were given six medical students who were instructed to turn over to us their final product for assay.

The following were the results: Student O obtained an inactive vaso-dilatin free product; Student K obtained the same results; Student Y, 15 doses, active in 6.1 mgm. doses, vaso-dilatin free; Student M, 17 doses, active in 5.9 mgm. doses, vaso-dilatin free; Student Q, 16 doses, active in 6.3 mgm. doses, vaso-dilatin free; Student F gave us a product which contained no activity, but much vaso-dilatin. Students O and K reported that they had trouble with step 7 and Student F, we believe, used the filtrate in step 2, but states that he did not. We cannot account for the results of Student F.

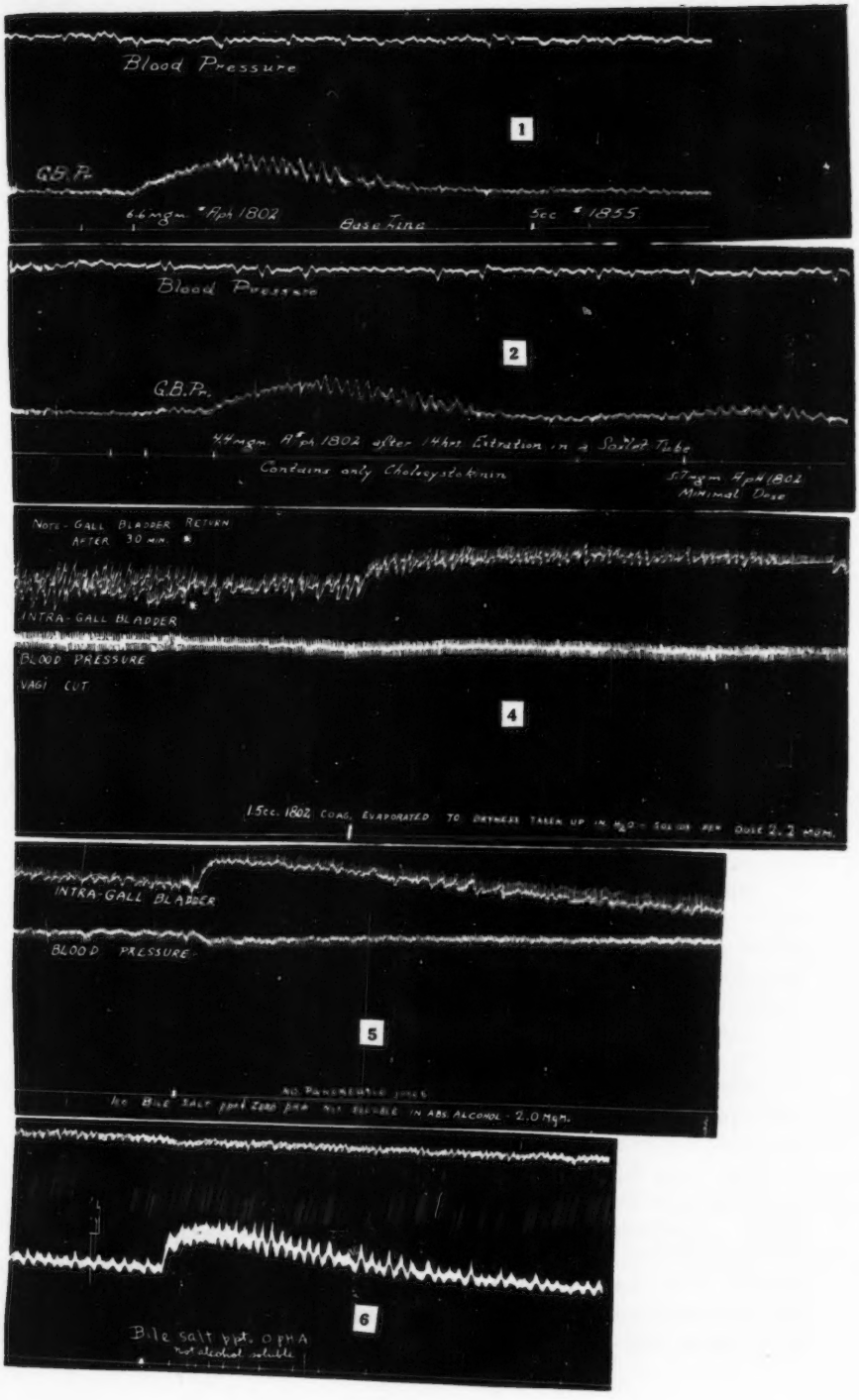
Fig. 1 shows a contraction of the gall bladder on the injection of 6.6 mgm. of "A pH 1802" powder.

Fig. 2 shows a contraction of the gall bladder on the injection of 4.4 mgm. of the "A pH 1802" powder used in figure 1 after 14 hours extraction with absolute alcohol in the Soxhlet. Note that this takes out impurity. This product contains no secretin.

Fig. 4 shows a contraction of the gall bladder on the injection of 2.2 mgm. of material made by the "1802 C and E" method.

Fig. 5 shows a contraction of the gall bladder from 2.0 mgm. of material prepared by bile salt precipitation. This material contained no secretin, the secretin being dissolved in the absolute alcohol.

Fig. 6 is a repetition of the procedure used in preparing the material used in figure 5, but this time it is observed that all the secretin was not soluble in the absolute alcohol.


Blood Pressure
1
G.B.Pr.
66 mgm. Apth 1802
Baseline
5cc. 1855
Blood Pressure
2
G.B.Pr.
44 mgm. Apth 1802 after 14 hrs. Extraction in a Soxlet Tube
Contains only Cholecystokinin
57 mgm. Apth 1802
Minimal Dose
NOTE - GALL BLADDER RETURN
AFTER 30 min.
INTRA-GALL BLADDER
BLOOD PRESSURE
VAGI CUT
4
1.5cc. 1802 COAG. EVAPORATED TO DRYNESS TAKEN UP IN H2O = SOLUTION PER DOSE 2.2 MGM.
INTRA-GALL BLADDER
BLOOD PRESSURE
5
6
Bile salt ppt. 0 PHA
not alcohol soluble

Figs. 1–6

The "S. III. pH 1802 method": Another reliable method which does not yield as pure a product as the "A pH 1802" method is as follows: 1. The sodium chloride precipitate obtained in step 3, which we call "A," is extracted with 95 per cent alcohol (room temperature). Any dilatin present, except that described by Ivy, Lueth and Kloster (1929), much of the secretin, and a small variable quantity of "cholecystokinin" is dissolved in the alcohol. Most of the "cholecystokinin" is insoluble. 2. The 95 per cent alcohol insoluble residue is taken up or suspended in water. This we call "S. III." Then NaOH is added to bring to a pH of 5.0 to 5.5. 3. Then the procedure described above is followed from step 6. The resulting product is active in from 10 to 15 mgm. doses.

Preparation "S. III.," which is crude, is a very standard and reliable product. It is always dilatin-free unless the "A" preparation contains a large quantity of "vaso-dilatin" due to using intestines that have not been obtained soon after slaughtering the hogs. It causes the type of vaso-depressor phenomena described by Ivy, Lueth and Kloster (1929), which can be avoided by isoelectric precipitation at a pH of 5.0 to 5.5, i.e., isoelectric precipitation removes the peculiar type of vaso-depressor material described by them.

"1810 method": A method which we thought at first had considerable promise was as follows: 1. The intestine was extracted with 0.5 per cent H_2SO_4. 2. The extract after one-half hour in the intestine was filtered and precipitated with trichloracetic acid (5 per cent as described above). 3. This precipitate was taken up in water, boiled and filtered clear, and then was evaporated to dryness. 4. The residue was extracted with ether, acetone, and absolute alcohol, the latter removing about 10 per cent of the secretin. 5. The residue was then extracted with 80 per cent alcohol which removes the secretin and variable quantities of "cholecystokinin." The 80 per cent alcohol soluble material on a number of occasions gave a "secretin" dosage in from 1.0 to 0.2 mgm. amounts and a gall bladder dosage in from 3.0 to 4.0 mgm. amounts. The method proved to be unreliable because sometimes the yield was 75 per cent and at other times no activity was obtained. We were unable to ascertain the exact cause. We believe that evaporating to dryness (75°C.) with some trichloracetic acid present to be the most probable cause, since we know that prolonged heating with dilute trichloracetic acid destroys activity.

"1802 C and E": The purest product that we have been successful in obtaining up to the present time was prepared as follows: 1. The sodium chloride precipitate, "A," was taken up in water and precipitated with trichloracetic acid (5 per cent). 2. This precipitate was taken up in hot distilled water, using as small a quantity as possible. 3. The solution was then boiled (only allowed to simmer) with a bumping stone for 20 or 30 minutes at the end of which period a heavy flocculent precipitate ap-

peared and increased on cooling. 4. This was filtered when cold, the filtrate being clear. 5. The clear filtrate was evaporated to dryness. 6. The residue was taken up in water, all of it not being soluble in water, some of the material having been denatured by the process of evaporation without loss of activity.

On one occasion we obtained a gall bladder response with 2.2 mgm. of this water soluble material. This method is unreliable because sometimes the yield is less than ten per cent and the amount of denaturization is variable which yields a product of variable purity.

Observations on stability: "Cholecystokinin" is destroyed by boiling with 10 per cent HCl for twenty minutes. Heating a solution when slightly alkaline with NaOH destroys it. It is soluble in warm glacial acetic acid and is not destroyed by such an extraction process.

A number of observations have been made on the relation of the pH of a purified solution containing cholecystokinin and its stability. At a pH of 5.0 it will keep in the ice box about one week, at which time the solution becomes cloudy and some precipitate is formed. The activity cannot be recovered by treatment with either weak alkalies or acids. At a pH of 4.0 the solution will keep from two weks to one month. At a pH of 3.0, the solution will remain active as long as five months. We have no results for a longer period.

The sodium chloride preeipitate ("A") shows no loss of activity when kept for a period of eighteen months. A solution of "S. III" (pH 2.0–3.0) has retained its activity for more than a year.

Bile salt precipitation: Because of Mellanby's (1928) success with "bile salt" precipitation of secretin, we tried this procedure on purified preparations of "cholecystokinin." Out of ten attempts, two were successful. In two other experiments the acid extract from the intestines was brought to a pH of 5.0. This gave a precipitate which was filtered. The filtrate was saturated with sodium chloride. The sodium chloride precipitate was taken up in water. Bile salt (Merck's sodium glycocholate) was added to this solution so as to have a concentration of 0.2 per cent; then sufficient glacial acetic acid was added to have an acidity of 0.2 per cent acetic acid. A precipitate resulted and increased on standing in the ice box (5°C.). This precipitate was collected in centrifuge tubes and extracted with absolute alcohol. The absolute alcohol insoluble residue contained gall bladder activity in 2.0 mgm. doses. We attempted to repeat this a number of times with negative results. We tried to control various factors such as the pH of the original solution, the amount of bile salt, and the amount of acetic acid added with no avail. In this connection we tried to adsorb it on various soaps with no success.

Adsorption to charcoal. We have a preparation of wood charcoal which will adsorb "cholecystokinin" from a solution of "A pH 1802," which

proved to be of no advantage because it did not result in purifying the solution.

Dialysis and ultra filtration: We have tried dialysis and ultra filtration through celloidin membranes. "Cholecystokinin" did not dialyze.

Other procedures: We have tried a great number of procedures involving the use of glacial acetic, phenol, phosphotungstic and molybdic acids, the heavy metals, brucine, salicylic acid, cholesterol, soaps, fractional precipitation with the organic solvents, continuous extraction with organic solvents, etc., without discovering a reliable procedure.

Sources: We have found "cholecystokinin" to be present in the upper intestine of the hog, dog, sheep and in cattle. A small amount can be extracted from the gastric mucosa, some attempts have been attended with negative results. The liver, kidney and spleen have been extracted without finding activity.

Egg yolk was also extracted without finding "cholecystokinin."

Is "cholecystokinin" different from secretin? We have several reasons for believing that "cholecystokinin" is not secretin. 1. There is a secretin which is soluble in absolute alcohol, and which never gives the "cholecystokinin" effect. If one extracts the intestinal mucosa with absolute alcohol, about 50 per cent of the secretin is removed, but the "cholecystokinin" is left, and can be extracted later with 0.4 per cent HCl (Mellanby, 1928; Mortimer and Ivy, 1929). Thus we have had solutions of secretin (they can be prepared routinely) which have contained no gall bladder principle. We have also had solutions of the gall bladder principle which contained no secretin. By the extraction of "A pH 1802 powder" which contains 3 or 4 doses of secretin for 1 dose of "cholecystokinin," in a Soxhlet apparatus with absolute alcohol, all the secretin is usually removed without loss of "cholecystokinin." This would constitute perfect proof provided we could state that we could recover all the secretin from the absolute alcohol. This we cannot do because the continuous heating of the secretin in the absolute alcohol destroys it. 2. Extraction of the intestine yields from 3 to 4 doses of secretin to one of "cholecystokinin." This ratio is not altered by precipitation with sodium chloride, nor by isoelectric precipitation (pH 5.0 to 5.5), nor by trichloracetic acid precipitation. At first we thought this disparity in dosage value might be due to the possibility that three or four doses of secretin were necessary to make the gall bladder contract, i.e., that there was a difference in the threshold of excitability of the gall bladder and pancreas to secretin. However, Ivy and Oldberg (1928) found in their cross-circulation experiments that the gall bladder responded to acid in the duodenum more readily than the pancreas did, which made the foregoing possibility untenable. Hence it is reasonable to conclude that the gall bladder response is not due to secretin but to some other agent.

Color reactions: Using our most highly purified products active in from

2.0 to 3.0 mgm., the biuret, Pettenkofer and the Pauly have been positive, and the Millon, the Molisch and the Hopkins-Cole have been negative.

DISCUSSION. Three principles are involved in the procedure which is most reliable, 1, precipitation of the active substance with sodium chloride; 2, isoelectric precipitation of impurity at a pH of 5.0 to 5.5, and 3, trichloracetic acid (5 per cent) precipitation of the active substance.

Considerable vaso-dilatin is present in all extracts of the intestinal mucosa, and the problem of prime importance is to find a procedure which will rid the material containing the active substance of vaso-dilatin without much loss of activity.

The principle of sodium chloride precipitation to rid intestinal extracts relatively free of vaso-dilatin was first discovered by Weaver, Luckhardt, and Koch (1926). They found that if an acid extract (0.4 per cent HCl) of the intestinal mucosa was precipitated by saturation with sodium chloride and then the precipitate washed with 0.4 per cent HCl saturated with salt, a product resulted which gave on intravenous injection a pancreatic response without a concomitant fall in blood pressure. We have found that their claim is true if dogs' intestines are used and provided one does not attempt to further purify and concentrate the sodium chloride precipitate. Also, we have found that the washing with 0.4 per cent HCl saturated with salt leads to some loss of activity, and if hogs' intestines are used, the sodium chloride precipitate always contains some vaso-dilatin. We tried to avoid this by taking up the sodium chloride precipitate in water or 0.4 per cent HCl and reprecipitating with salt. We met with difficulties in this procedure, in that it sometimes worked, but with a resulting loss of approximately one-third the activity, and that sometimes perfect precipitation did not occur, the filtrate being milky. If hogs' intestines are used, even if one is successful in getting rid of the "histamine type" of vaso-dilatin by two precipitations with salt, a second type of vaso-depressor activity remains in the solution (Ivy, Lueth and Kloster, 1929). The principle of isoelectric precipitation is of distinct advantage, because it rids the material of much protein impurity and of the vaso-depressor material just mentioned. The principle of trichloracetic acid precipitation has the advantage that it rids the material of most of the salt and the remainder of the "histamine type" of vaso-dilatin.

We believe that the "A pH 1802" method is a good one because it is simple and reliable, which is shown by our adoption of it as a uniform procedure and by the results of its use by medical students without supervision.

The principle of "bile salt" precipitation would be excellent provided it were reliable. This principle in our hands has met with more failures than successes, which was also true when it was used for the isolation and purification of secretin (Mortimer and Ivy, 1929).

The evidence presented on the question, "Is 'cholecystokinin' different from secretin," is sufficient, we believe, to have warranted the coining of the term "cholecystokinin." Our opinion is that "cholecystokinin" is not secretin, but is closely allied to it.

SUMMARY

1. A method is outlined for the preparation of "cholecystokinin," which yields a uniform product containing from 3 to 4 doses of secretin for one of "cholecystokinin," free of vaso-depressor action, and causing gall-bladder contraction in from 3 to 6 mgm. doses (from dog's mucosa) and in from 5 to 10 mgm. (from hog's mucosa) doses, when administered intravenously.

2. We can make a solution which contains secretin but no "cholecystokinin," and a solution which contains "cholecystokinin," but no secretin. This observation causes us to conclude that "cholecystokinin" is not secretin.

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EVIDENCE THAT THE POSTURAL TONUS OF DECEREBRATE RIGIDITY INCREASES IN AMOUNT BY THE SUCCESSIVE INNERVATION OF SINGLE MOTOR NEURONES

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The trend of recent papers has been against the view that postural tonus[1] is produced or maintained by the sympathetic nervous system or by any special musculature. Instead the opinion seems to be growing that it results from the contraction of ordinary striated muscle controlled by the central nervous system through the usual channels (cf. Tower, 1929). I wish to report some observations which tend to support this latter point of view. Certain resemblances will be shown between the flexion reflex of the spinal cat and tonic contraction as seen in the decerebrate cat. In particular it will be shown that in decerebrate tonus if a sufficiently small muscle be used, evidence can be seen of the activity of single motor neurones coming into play, one after another, gradually increasing the height of the tonic contraction. In spinal reflex flexion the activity of single motor-neurones can be similarly demonstrated (Porter and Hart, 1923).

It has been recognized for some years now that with a little care in registration the contraction of the muscle fibers innervated by a single motor-neurone could be registered. Mines (1913) obtained such records for the nerve-muscle preparation of the frog with ordinary smoked drum registration. The record increased in height with increase in stimulation by a series of steps, each step consisting of several contractions of equal height. The accepted explanation is that each step represents the contraction of all the muscle fibers innervated by a single motor-neurone. A series of such steps results as the current strength increases gradually to the threshold of stimulation of one motor-neurone after another. The phenomenon is a relatively easy one to demonstrate and may be seen at times even in the student laboratory.

Hart and I (1923) showed that the same phenomenon could be demonstrated for reflex contraction. A minute muscle, the tenuissimus, in-

[1] The word tonus will be used in this paper as a convenient short equivalent for "the postural contraction of muscle as seen in decerebrate rigidity."

volved in the flexion reflex in the spinal cat, was used and gradually increasing shocks were applied to the proper sensory nerve. Contraction was isotonic. Here sensory neurone, cord and motor-neurone were involved in contrast to the motor-neurones alone as in Mines' (1913) experiment. But the same steplike character of the contractions resulted and the steps were of approximately the same height as the nerve-muscle steps from the same muscle. It was argued, therefore, that in reflex flexion increasing sensory stimulation had called into action one motor neurone after another just as increasing shocks to the motor nerve had done in the nerve-muscle preparation.

Using much larger muscles and isometric recording Liddell and Sherrington (1924) have shown that reflex increase in tension shows the same evidence of successive activity of single motor-neurones. They have given the name "recruitment" to the phenomenon, and Sherrington (1925) proposes the term motor-unit for the motor-neurone with the muscle fibers innervated by it. This supplies a brief term which has been much needed. It is unfortunate that we have no term which would indicate a little more definitely the complex character of this unit since each motor-neurone innervates presumably one hundred to one hundred and fifty fibers (Adrian, 1925). Such a term, for example, as "nerve-muscle squad," on the analogy of a corporal's squad of an army would carry that idea.

In view of the wide disagreement in recent years in regard to the whole subject of tonus it has seemed desirable to learn if possible whether the activity of single motor-unit or "nerve-muscle squad" could be detected in tonic contraction as Hart and I (1923) observed the phenomenon in the spinal reflex. Would increase in height of tonic contraction occur in steps and if so would the steps be of approximately the height of those obtained from the nerve-muscle preparation, using the same muscle? If so it would indicate the use of the same mechanisms as those employed in producing the steps in spinal reflex contraction height. If on the other hand the record of tonus should show a smooth slope as tonus increased in amount, this would indicate a profound difference in mechanism from that of the spinal reflex or the nerve muscle preparation.

Using a quite different method from mine Denny-Brown (1929) has investigated the stretch reflex in the decerebrate animal on the basis of recruitment of motor-units. He registers the action current from the muscle during isometric contraction. His evidence for the successive entrance into activity of individual motor-units in this reflex is very convincing. His records are obtained during continuation of the stretch of the muscle and for brief periods. The data of the present paper, in contrast, were obtained during long-continued and self maintained tonus using isotonic contraction height to register recruitment and the nerve-muscle preparation to give the size of single motor-unit contraction. The

method is relatively crude compared with the refinements of the Sherrington method, but it is a part of the purpose of this paper to point out how comparatively easily, with simple apparatus, the activity of single motor-units in postural tonic contraction can be demonstrated.

METHOD. To demonstrate steps in muscle response by isotonic registration a very small muscle is needed. Only the first few steps, near the threshold, can be demonstrated clearly and in a large muscle the activity of the muscle fiber group innervated by a single neurone is unable to register its activity on account of the mass of inactive fibers in which it is imbedded. Only in a small muscle is the activity of a single "neuro-muscular squad" able to move the entire muscle in spite of the inertia of the inactive fibers around it. The tenuissimus in the cat is quite small enough to demonstrate the phenomenon for the flexion reflex, but amongst the leg muscles taking part in decerebrate rigidity there is none remotely approaching the small size of the tenuissimus. Certain of the tail muscles were however found to be sufficiently small.

Elevation of the tail is a part of the picture of decerebrate rigidity, although by no means as constant as extension of the legs. The small tail muscles are not separate but are united into a long, narrow sheet of tissue known as the extensor caudae lateralis. The subdivisions correspond to the vertebrae and each has a separate tendon. The origin of each subdivision is from the transverse process of a caudal vertebra and the insertion is some fifteen vertebrae farther down the tail on the dorsal surface of a vertebra.

By selecting one tendon the contraction of a sufficiently small segment of the whole muscle can be successfully registered. The small muscle connected with a single tendon is roughly comparable in size, shape and proportions to one's little finger-nail.

The preparation of a cat for this work is as follows:

It is decerebrated by the Pollock-Davis method (Pollock and Davis, 1923). This involves the tying of both carotids and the trephining of a small hole about an eighth of an inch in diameter through the roof of the mouth. The basilar artery is thereby exposed and in the original method was ligated.

I have adopted, however, the easier procedure of applying a small clip to the artery. At times I have merely ruptured the artery either accidentally or purposely according to Ranson and Hinsey's (1926) modification and found this procedure to be successful. The hemorrhage is not great upon packing the mouth with muscle and cotton. An animal can thus be saved in case of accident in applying the clip. The clip I have used is of spring brass wire made like a small bull-dog clamp 1 cm. long and is applied with forceps. Upon applying the clip, the portion of the brain forward of this point is rendered completely anemic. Within a few

seconds, unless the animal be too deeply anesthetized, typical decerebrate rigidity commences and is speedily complete. The tail is usually involved as Pollock and Davis (1923) have reported. In my series of animals the tail failed to show extension in only two cases out of thirty-six. Producing a decerebrate animal by this method seems somewhat formidable upon first reading, but the method is not difficult to acquire. In my own experience, only three or four animals were necessary before the procedure could be carried out with routine success. A foot power dental engine was employed instead of the motor driven apparatus of Pollock and Davis and a vulcanizing burr about an eighth of an inch diameter was used for the trephining. A desk lamp supplied sufficient illumination of the mouth.

I believe the method to be superior to other methods of decerebration where animals are to be used for some hours for experimental purposes and particularly when the tail is to be used. At times, as with other methods, artificial respiration is necessary for a brief period.

Fig. 1. Clamp for immobilizing the base of the tail. See description in text.

The cat is supported in a special cradle of wood modelled to fit the body. The legs hang freely, clearing the table by an inch or so.

In registering minute contractions from the tail muscles, it is of importance that movements of respiration be prevented from influencing the lever. This is not an easy matter. The clamp shown in figure 1 gave the best and quickest immobilization. This is in the form of a split fork with the tines sharply pointed and is applied in such a way as to grip the vertebral column above and below at the level of the sacral and lowest lumbar vertebrae as shown in the figure. In applying it, the skin is incised on the right side over the sacral and upper caudal vertebrae for a distance of 5 or 6 cm. The muscles and fascia are cut through opposite the sacrum, and the lower half of the fork is thrust through this opening in a direction parallel to the vertebral column on its ventral surface and as closely as possible to it. It penetrates the mass of the psoas muscle. The other portion of the clamp is applied to the dorsal side of the vertebral column, being thrust into the muscle-mass to the side of the spinous processes.

The two portions of the clamp are then screwed together in such a way as to enclose between their halves a flat piece of galvanized iron about four inches long which is screwed solidly to the cradle in which the animal lies. This piece of iron is turned up at the edge slightly on the side toward the tail to prevent lateral movement of the clamp. Upon tightening the

clamp, the vertebral column becomes solidly attached by means of the clamp to the piece of iron and thereby to the cradle. Rigidity is destroyed in one hind leg through the application of this clamp, but the animal is otherwise not affected. The clamp effectively prevents ordinary movements of respiration from being registered on the drum. If the animal is breathing deeply, this is not true, but the undulations on the record are usually so small and regular that they do not confuse the results.

The tail is supported horizontally on a narrow extension of the cradle and the muscle is prepared for registration as follows:

After clipping the hair on the dorsal surface, the skin is incised for 3 cm. at a point about midway of the tail. The numerous tendons which act to elevate the tail are thereby exposed. One is selected which comes from a portion of the muscle in the neighborhood of the clamp. This can be determined by inspection upon pulling on the tendon. A silk thread is tied about the tendon and the tendon cut distally. The thread is carried to one arm of a light straw writing lever, 40 cm. long. The arrangement is such that magnification is about forty times. No weight is applied to the lever, the weight of the straw being sufficient. This gives a tension on the muscle of approximately 5 grams. Registration is on a slow drum, moving usually about 2 cm. a minute.

If nerve-muscle records are to be secured, the skin and fascia are incised over the muscle to be used. By pulling the muscle-mass slightly to one side with a clip the motor nerve is exposed. This is so small as to make the isolation of the nerve itself impractical, and its stimulation is brought about by placing an electrode in direct contact with it as it lies on the surface of the muscle. The electrode used has been a very simple one composed of a piece of spring brass wire coiled loosely in half a dozen turns an inch or so in diameter and pointed sharply at the lower end. It is held suspended in a clamp and the point applied delicately to the muscle. By exploring with this point over the surface of the muscle, employing weak tetanizing shocks, contact with the nerve is made evident by the sudden contraction of the muscle. The indifferent electrode is the clamp on the vertebral column. A mercury key is employed, whose point is amalgamated before each experiment; otherwise the stimulating outfit is such as is usually supplied to students.

Results. Since the conclusions which are to be drawn with regard to tonus depend on the character of the nerve-muscle records these will be described first. A typical nerve-muscle record is shown in figure 2. The point of the spiral wire electrode was resting lightly on the surface of the muscle at the point which by search had given the sharpest contraction with weakest stimulus. Break shocks were employed. Beginning with a shock which caused no contraction the strength was gradually increased by the simple expedient of tapping the secondary with a pencil after each

stimulus to turn it more nearly into line with the primary. Shocks were increased to the center of the record and then decreased until no contraction occurred. Three steps in contraction height are plainly distinguishable indicating the activity of three motor-units. The staircase phenomenon seen here is often but not always present.

Other nerve-muscle records are shown in each of the figures which follow. Small undulations in the base line when present are due to respiratory movements unavoidably transmitted to the tail. Allowance must be made for such undulations in estimating contraction heights.

In most cases in securing these nerve-muscle records the motor nerve has not been cut between muscle and cord. To cut the nerve ends the experiment, so far as tonus is concerned, and yet it is always desirable to make an immediate comparison of nerve-muscle and tonus steps on the record before conditions change. If the nerve is not cut, however, there is the possibility that reflex effects are entering in; a step in contraction height, for example, might be made up of several "neuro-muscular squads" in activity instead of one as in a typical nerve-muscle record. This would make it impossible to draw conclusions depending on comparing tonus steps with nerve-muscle steps. On theoretical grounds one would expect that near the threshold of stimulation reflex effects would not appear even though the nerve were not sectioned, for it has been shown that nerve-muscle thresholds are always lower than reflex thresholds (Sherrington, 1906; Porter, 1912). Actual experimental evidence was desirable, however, as to the similarity of records before and after cutting the motor-nerve, and this has been obtained in a number of animals.

The nerve has not been cut at the muscle, which would be a difficult matter on account of the small size of the nerve and the difficulty of locating it by sight, but by section of the cauda equina at the level of the last lumbar vertebra.

Inspection of pairs of records thus secured shows that they are essentially alike. Such a comparison is shown in figure 3. The increments in contraction height are distinctly of the same order of magnitude. The two records are from the same muscle with as little shifting of the electrode as possible. Some shifting is inevitable in view of the violent contractions of the muscle as the nerve is cut. Often the electrode is thrown from its first position and has to be completely readjusted; exact duplication of records therefore cannot be expected. See also in this connection the two records of figure 2 where the second nerve-muscle record was secured seven minutes after the heart had stopped and when reflex action was, of course, no longer possible.

This similarity in record before and after cutting the motor-nerve has been confirmed in eight animals. It has therefore been regarded as a justifiable conclusion that in those cases where the motor nerve is stimu-

lated without being cut the resulting contractions are not reflex but nerve-muscle, and that each step is the expression of the activity of a single motor-neurone and its attached muscle fibers.

These facts having been established the original question presented itself: would changes in tonus show evidence of the individual activity of these nerve-muscle units or would the tonus mechanism be able to overcome the restrictions of the nerve-muscle mechanism and give a smooth curve of increase or decrease in contraction height? The evidence to be reported indicates that tonus does not overcome these restrictions but calls on one neuro-muscular unit after the other for augmentation of tonus, the resulting record having a steplike character due to the all-or-none contraction of each muscular group.

This cannot be demonstrated on every animal; the condition of tonic contraction in the tail muscles varies too much to make this possible. Decerebrate tonus cannot be controlled at will as can the flexion reflex in the spinal cat, and it is more variable in the tail than in the limbs. This variation is not only from animal to animal, but in the same animal at different times in the same experiment. It may be too strong or absent, elicitable at one moment and not the next, appear spontaneously or only in response to stimulation. In all but two cats out of thirty-six decerebrated by the Pollock-Davis method it was present at some time or other during the experiment, but not always in usable form. The best records are secured when the tail muscles do not show tonus of themselves but when the mechanism is so delicately poised that it can be thrown into activity easily.

When an animal is in this condition one of the most effective forms of stimulation is the rubbing of the tail fur toward the head. Sometimes the rubbing has to be vigorous, sometimes the merest touch is effective and conditions often change completely in the space of a few minutes.

A record obtained by this means is shown in figure 2. First there is the record of nerve-muscle contractions, obtained in the usual way, to indicate the size of nerve-muscle increments in this particular animal. This was followed immediately by stimulation of the tail by rubbing the tail fur. The muscle responded at first by single twitches, unsustained, and then by a contraction sustained for two minutes without stimulation, a condition of tonus in the sense the word is used in this paper. It will be noticed that the single twitches at the start are of the same general height as the contraction of the nerve-muscle record and that the height at which contraction is sustained is that of one or possibly two of the steps. The experiment was repeated by rubbing the tail fur between *6* and *7*. Twitches were again followed by sustained tonus. The tonus is rather clearly of two nerve-muscle steps here, one of them partially dropping out of action after about forty seconds.

The tonus of figure 2 is only one or two steps high indicating the innervation of only one or two motor-neurones. In other animals it has been possible to produce a tonus many steps in height, each step perfectly sharp. An example is given in figure 3. (See also fig. 6.) The tail fur was rubbed between *1* and *2*. A tonus of one step resulted. Momentary rubbing of tail fur at *3* and *4* gave successive sharp steps all of the same general size as the nerve-muscle steps shown in the preliminary record. Often such tonus is very persistent and prevents further experimentation.

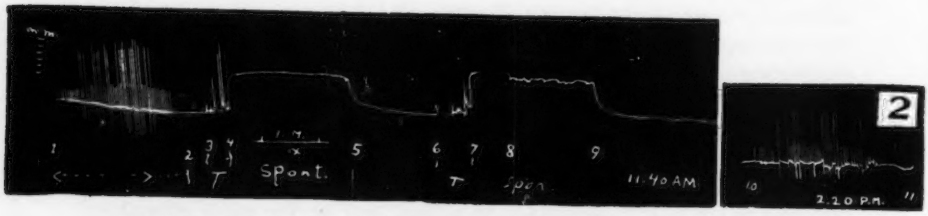

Fig. 2. Decerebrate cat. Extensor caudae lateralis muscle. The small portion attached to one tendon.

1–2, Nerve-muscle record, spiral wire electrode on motor point of nerve. Shocks increased and decreased between *1* and *2*. Note three steps indicating three motor units coming into action.

3–4, Tail fur rubbed toward the body for about 30 seconds. Twitches occur at heights corresponding to the nerve-muscle steps secured between *1* and *2*. At *4* the muscle went abruptly into spontaneous tonus at a height corresponding to one or possibly two of the nerve-muscle steps.

5, Tonus ends. *6–7*, Tail again rubbed causing twitches at height of nerve-muscle steps as before.

7–9, Abrupt self-maintained tonus (stimulation discontinued) at apparent height of two nerve-muscle steps. At *8* one step—one motor unit—shows fatigue, passing in and out of action. At *9* a sudden return to the original base line.

10–11, Nerve-muscle record 7 minutes after heart had stopped beating from asphyxia (illuminating gas). Hence no reflex action possible. Shocks increased, then decreased. Note correspondence in steps to those of first nerve-muscle record indicating that the first record was pure nerve-muscle without reflex admixture. Approximate duration of 1 minute shown at *X*.

Record natural size. Lever magnification *ca* 40×. Tension on muscle about 5 grams. Isotonic contraction. (March 26, 1927.)

In this case, in order to return the muscle to rest, the tonic contraction was terminated by the administration of ether at *5*. The first effect was a stimulation with an increase in tonus, then a relaxation of the muscle to resting level. After a lapse of several minutes tonus was again produced in this animal in the same way by rubbing the tail fur and again lowered by ether.

The process of securing a nerve-muscle record has at times produced tonic contraction. In the experiment shown in figure 4 after a few twitches

caused by stimulation of the motor-nerve the muscle did not relax to the resting level as before but suddenly passed into tonic contraction which was at the height of a single nerve-muscle step. Further stimulation resulted in a repetition of the phenomenon starting from the new level. The record suggests that we have here a proprioceptive reflex in its simplest form.

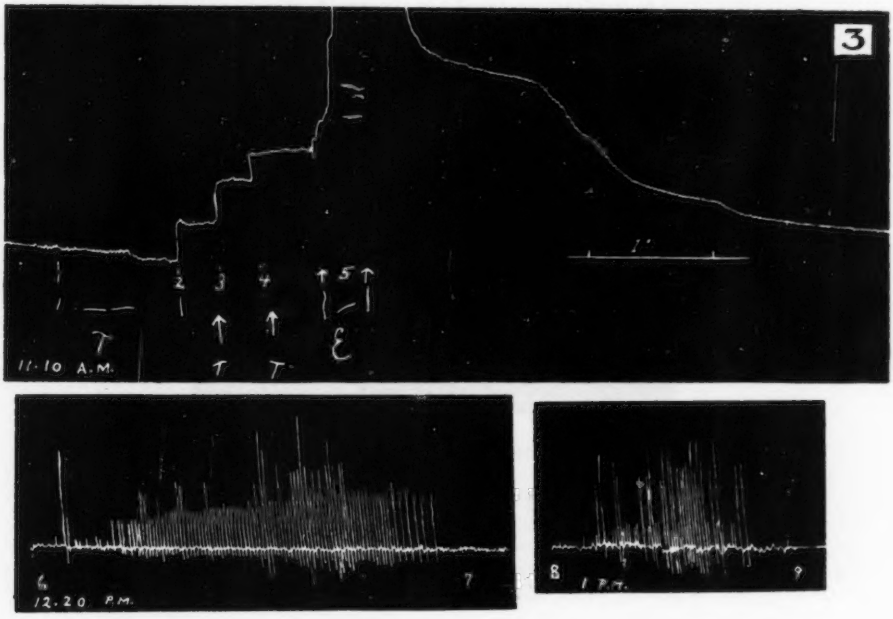

Fig. 3. Decerebrate cat. Single tendon portion of extensor caudae lateralis muscle.

6–7, Nerve-muscle record. Motor nerve uncut. Shocks increased then decreased.

8–9, Nerve-muscle record. Motor nerve cut. Note correspondence in height of steps with those of the previous record, indicating that a nerve-muscle record made without cutting the motor-nerve is probably a valid nerve-muscle record without admixture of reflex elements.

1–2, Tail fur rubbed toward head continuously.

3 and *4*, Tail fur rubbed toward head momentarily. Following each stimulation tonus increased by a step-like increment, indicating activity of successive motor units. The steps are of approximately the same size as those of the nerve-muscle records.

To indicate that this was a true tonus of central origin ether was administered at *5* to relax the tonus. This it did although the first effect was a stimulation with increase in tonus.

The small waves are respiratory in origin. Record natural size. Lever magnification *ca* 40×. Isotonic contraction. Tension on the muscle about 5 grams. The approximate duration of one minute is shown under tonus record. (July 10, 1928.)

We may imagine a single sensory end organ (Adrian and Zotterman, 1926) stimulated by the fibers contracting in the twitch, acting reflexly on a single motor-unit.

Sometimes in the absence of any detectable stimulation the muscle will spontaneously pass into tonus. The record always shows sudden step-like increments in contraction height of the same order of magnitude as the nerve-muscle steps. Such a record of spontaneous tonus change is shown in figure 5. The nerve-muscle record is appended. The steps in the tonus record which are significant and which are to be compared with the nerve-muscle steps are the sudden ones with an almost vertical rise. The smaller

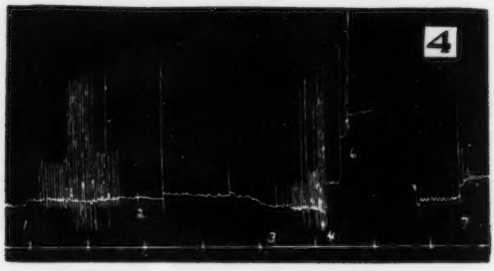

Fig. 4. Decerebrate cat. Extensor caudae lateralis muscle—small portion acting on a single tendon.

1–2, Nerve-muscle record. Shocks increased then decreased. Two steps indicating two motor units successively brought into action.

3–4, Attempt to repeat nerve-muscle record.

At *4* tonus appeared and was sustained spontaneously for 15 seconds at about the height of the first step; again at *5* for a few seconds at a second step height and at *6* for 30 seconds at a third.

At *7* spontaneous twitches appeared again followed as before by tonus held at the level of the first step.

The small waves are respiratory in origin transmitted unavoidably to the tail.

Record natural size. Lever magnification *ca* 40×. Time in minutes. Isotonic contraction. Tension on the muscle about 5 grams. (June 30, 1927.)

undulations are from respiratory and other movements in the forward part of the body which could not easily be eliminated from the record.

In no case of spontaneous increase in tonus are these sharp steps absent and they are always of approximately the height of some nerve-muscle step from the same muscle. The slope upward is never a smooth one. The evidence seems strong that this is a process of recruitment (Liddell and Sherrington, 1923) of single motor-units.

As tonus subsides step-like drops are often to be seen as in figure 6. The level at *9* had been produced by rubbing the tail fur in the manner already described. This had been repeated in this animal a number of

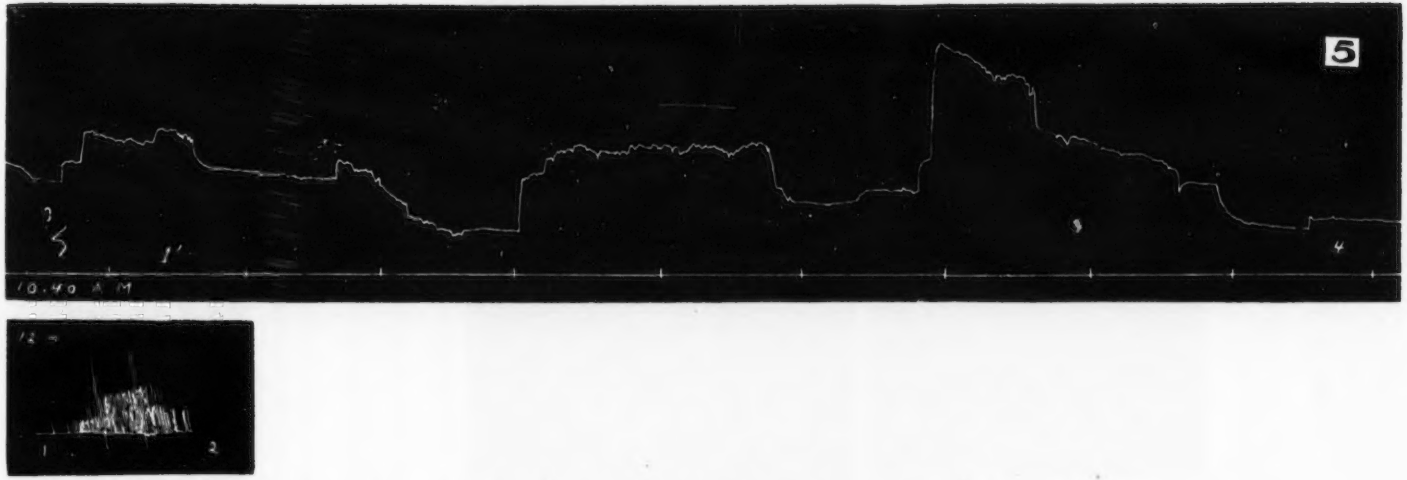

Fig. 5. Decerebrate cat. Extensor caudae lateralis muscle. Minute portion of the muscle attached to a single tendon.

1–2, Nerve-muscle record. Shocks increased, then decreased. Two steps definitely shown indicating the activity of two motor-units.

3–4, Spontaneous alterations in tonus; no known stimulation. Abrupt steplike increases in tonus, often closely corresponding to the nerve-muscle steps. Decrease in tonus is also by steps at times but at other times the descent is gradual. See discussion, p. 354.

(The smaller waves in the record are not contractions of the tail muscle, but are respiratory and other movements transmitted unavoidably from the forward part of the animal.)

Record natural size. Lever magnification *ca* 40×. Isotonic contraction. Tension on the muscle about 5 grams. Time shown in minutes. (July 26, 1927.)

times. One of the characteristic series of steps on the upgrade is shown between *3* and *7* of this figure. Between *9* and *10* over a period of several

Fig. 6. Decerebrate cat. Extensor caudae lateralis muscle, portion attached to a single tendon.

1–2, Nerve-muscle record; shocks increased, then decreased. Three steps definitely discernible indicating three motor-units.

At *3*, *4* and *5*, tail fur rubbed toward the body momentarily each time. Step increase in tonus.

6–7, Tail fur rubbed continuously. Further step increase in tonus.

7–8, Tonus level maintained without stimulation, dropping eventually nearly to original level. Some steps descernible.

9–10, Tonus previously produced by rubbing tail fur diminished by steps. Note correspondence to certain steps of the nerve-muscle record. The diminution of tonus by steps indicates presumably the dropping out of action of one motor-unit after another.

The small waves are respiratory in origin, the movement being transmitted to the tail.

Record natural size. Lever magnification *ca* 40×. Isotonic contraction. Tension on the muscle about 5 grams. Approximate duration of 1 minute shown on one record. (June 11, 1928.)

minutes the tonus subsided. Definite steps are present pointing to the withdrawal of successive motor-units from action.

In these records of the subsidence of tonus the steps are seldom as sharp as those of the upgrade. This is perhaps to be expected. We may imagine that in the diminution of tonus, fatigue may be both central and peripheral. If it is in part peripheral, some of the muscle fibers involved in the contraction probably go out of action before others in the same "squad," thus rounding off the angle of the step. When central fatigue occurs the entire squad goes out of action and the sharper drop occurs. When called into action, on the other hand, all muscle fibers being un-

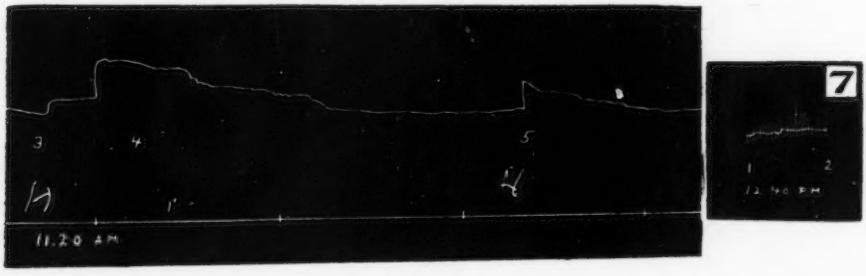

Fig. 7. Decerebrate cat. Small portion of the extensor caudae lateralis muscle acting on a single tendon.

1–2, Nerve-muscle record. Strength of shock unchanged. Activity of two or possibly three motor-units is indicated.

3–4, Head was slowly and steadily raised with strong dorsi-flexion of neck. Steps correspond roughly in height to the heights of contraction in the nerve-muscle record.

At *4* the head was allowed to return to the horizontal position. Steps are only roughly indicated in the relaxation which follows, but the fatigue is perhaps in part peripheral (see p. 354).

At *5* an attempt to repeat the experiment resulted in one step of tonus only.

In this animal moving the foot caused the typical tonic contraction also. (June 29, 1927.)

fatigued, the entire squad would come into action on an all-or-none basis, giving the sharp steps of the upgrade.

Movements of the head, particularly strong dorsi-flexion of the neck were sometimes effective in arousing the tail muscle to action when other stimuli were not. An example is shown in figure 7. The head was slowly and steadily raised at *3* and lowered at *4*. Two steps of tonus resulted. But there was no certainty about this reaction any more than in the case of any other stimulus, and in the same animal it was sometimes effective, sometimes not. Such head movement combined with rubbing the tail fur was at times effective when either alone was ineffective.

At times tonic contraction of the tail muscle of the usual step-like character would be aroused by quite unusual and unexpected stimuli

TABLE 1

DATE	METHOD OF DECEREBRATION			CAUSE OF TONIC CONTRACTION OF TAIL MUSCLE*				SPONTANEOUS TONIC CONTRACTION OF TAIL MUSCLE*
	Clip on basilar artery	Rupture of basilar artery	Trephine or guillotine	Rubbing tail fur headward	Head up, dorsiflexion of neck	Tapping on stand	Unusual causes	
1927								
3/2	X							XX
3/14 (a)		X			X			X
3/14 (b)	X							X
3/19	X						Ether	
3/22		X		XX			Ether	X
3/26	X			XX				XX
6/16	X			XX				X
6/18	X							X
6/22		X					Moving hind leg	
6/24 (a)		X						
6/24 (b)			G	XX			Jarring table	XX
6/29	X			X	XX		Moving foot	X
6/30	X			X	X			
7/12	X				X			
7/15	X			XX	XX			XX
7/20		X		XX	XX			XX
7/22	X			X	X			X
7/26		X		XX				XX
8/9	X							
8/11	X							
8/12	X			XX				XX
8/15	X							X
8/16	X			X				
1928								
6/4		X			X	X		
6/6		X		X				
6/7	X							X
6/11	X			XX				XX
6/13	X				XX	X		
6/15			T	XX				
6/19	X				X			X
6/21	X			XX				X
6/24	X			XX	X		Whiskers touched, nose rubbed	X
7/3	X			XX	X			X
7/5	X			XX	X		Ether	
7/7	X			XX				
7/10	X			XX			Ether	

* X, tonus present; XX, tonus especially well shown.

such as moving the leg, pulling the whiskers, etc. These are listed in the table on page 358.

In some animals the rigidity of the tail has been so persistent and extreme that it has been necessary to etherize the animal in order to reduce the contraction to a usable amount. Results of this procedure are various. Sometimes the tail rigidity disappears, never to return, or it may disappear and then return so suddenly and strongly that it cannot be studied. Often the first effect is an increase in tonus (see fig. 3). In successful cases tonus will disappear and then as the ether wears off will show for a time a stage in which it can be very easily evoked and studied. As the ether completely wears off, tonus is likely to return in full force making further experimentation impossible for the time being.

It should be noted in general that the widest variation in tonic activity of the tail muscles may be present in the same animal over a period of several hours. In some instances an animal has been set aside as useless for the time being and is then returned to after several hours, when entirely successful results will be obtained.

Table 1 is a summary of the results of the entire series of experiments which were performed after the technique of decerebration by the Pollock-Davis method had been mastered. This table is included to indicate what measure of success may be expected when the tail muscles are used for problems of this character.

Discussion. The chief purpose of this paper has been to describe the evidence which indicates that when the nervous system causes an increase in postural tonus, it calls on single motor-neurones one after the other and that in this ability to utilize single motor-units it resembles spinal reflexes. The evidence favors the view that the same fundamental mechanisms are employed in both cases; viz., striated muscle innervated through the usual channels of the central nervous system. It is beyond the scope of the paper to go further than this into the nature of tonus.

The records described have, however, a direct bearing on one of the phenomena of postural tonus, viz., its supposed lack of fatigue. If we regard the behavior of the single motor-unit as it takes part in postural tonus as in figure 2, for example, it is evident that to say that it does not fatigue has no meaning. The motor-unit acts and then with no outside interference ceases to act. The only word we have for such cessation of activity is fatigue. The second tonic contraction of figure 2 is interesting in this connection. The second record is apparently made up of two units—two steps—a small and a large. At *8* the smaller unit begins to fatigue. The fatigue is intermittent; the unit goes into and out of action momentarily for longer or shorter intervals. At *9* both motor-units succumb to fatigue and the record drops back to the original resting level.

Forbes (1922) has suggested a rotation of activity of nerve-muscle squads or motor-units as the reason for the apparent lack of fatigue in tonus.

Lingle (1910) had earlier suggested this for the tonus of smooth muscle and Barbour and Stiles (1912) for reflex action. Denny-Brown (1929) sees no evidence of such alternation of motor units in his records of action-currents in the stretch reflex and hence is inclined to reject Forbes' theory. Denny-Brown's records, however, were over comparatively brief periods. Longer records seem necessary to show the development of fatigue and its recovery. The rotation of motor-units seems to be definitely indicated in such a record as that of figure 5 in the present paper. In spite of the undulatory character of the record the general contraction height is being maintained at a more constant level than is apparent at first sight. The lever magnifies muscle movement forty times and the record is from only one of the fifty or more similar muscles elevating the tail. If we should watch the entire tail held up by all fifty muscles we should probably see scarcely a tremor even though each muscle were undergoing fatigue of the character indicated in figure 5.

As one inspects the records of the behavior of these single motor units as they undergo tonic contraction one questions whether tonus should not be regarded merely as a special case of reflex after-discharge. In figure 2 rubbing the tail fur for a few seconds threw one or two motor units into tonic contraction lasting two minutes. To follow Sherrington's (1906) description of after-discharge, the moment of cessation of stimulus and the moment of cessation of end effect did not correspond. The tonic after-discharge is long, that of a spinal reflex short; is that after all the chief difference? To say, of course, that this tonic contraction is merely after-discharge does not necessarily simplify the explanation of its causation and maintenance. All it does is to place it in the category of a phenomenon already long recognized in spinal reflexes; but just how a reflex center operates in bringing about after-discharge remains a problem.

The conclusions drawn in this paper depend upon the ability to demonstrate and study the behavior of single motor-units of reflex activity. It seems to the writer that the possibility of studying reflexes from the standpoint of the single motor-unit has not been sufficiently recognized. The desirability of using these ultimate units in many problems is self-evident; otherwise delicate phenomena may pass unnoticed. When we are using a massive muscle and a massive reflex we are in somewhat the same position we should be in if in watching the maneuvers of an army we should pay no attention to the management and behavior of a corporal's squad. The movements of the squad may be quite different from that of the army as a whole. Fatigue of the squad, for example, may be frequent and complete while fatigue of the entire army may seldom or never occur. Fatigue may seem to be absent in tonus; but tonus in the single motor-unit shows fatigue indubitably.

SUMMARY

1. A method is described by which a small muscle in the tail is used in the study of postural tonus in the decerebrate cat.

2. When this is used as a nerve muscle preparation and gradually increasing single shocks are applied to the motor-nerve, the response of the muscle is by contractions occurring in steplike groups with no gradation between successive groups. Each group step represents, presumably, the activity of a single motor neurone and the muscle fibers innervated by it.

3. Tonic contraction may be induced or increased in the tail muscle by various measures which are described, or it may occur spontaneously. By whatever means produced the increase in contraction height is never smooth and uniform but always by a series of sharp steps.

4. The steplike increments in tonic contraction height are approximately the same size as the steps in a nerve-muscle record from the same muscle.

5. The inference is drawn that in producing an increase in postural tonus the central nervous system throws into action one motor neurone after another. The all-or-none contraction of all muscle fibers innervated by each neurone produces the steplike increase in contraction height.

6. In certain records when the tonus is spontaneous and self-perpetuated, steps may be seen to drop out from the record and reappear again while the general level of contraction remains fairly constant. This is taken as evidence of rotation of activity of single neuromuscular units of contraction. This supports the theory that the apparent lack of fatigue in postural tonus depends on such rotational activity (Forbes, 1922).

7. The fact that steps of an all-or-none character corresponding in size to nerve-muscle steps may be demonstrated both in the spinal flexion reflex and in postural tonic contraction is regarded as favoring the view that the same mechanisms on the motor-side are operative in both cases.

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HYDROLYSIS OF PHOSPHOCREATINE AND LACTIC ACID PRODUCTION IN FROG'S MUSCLE

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In their 1927 communication on the labile phosphorus in cat's muscle Fiske and Subbarow (1) reported that there was in voluntary muscle a compound of phosphoric acid and creatine which underwent hydrolysis during muscular contraction, and which was resynthesized when fatigued muscle was allowed to recover. Calculations based on the dissociation constants of this compound lead them in their 1928 communication (2) to suggest that the liberation of base by the hydrolysis of phosphocreatine "is particularly designed for the neutralization of the lactic acid formed in muscular exercise of a moderate intensity." Meyerhof and Lohmann (3) had claimed that in frog's muscle the breakdown of phosphocreatine during brief periods of stimulation proceeds more rapidly than the production of lactic acid; i.e., in the first 5 seconds of tetanus twice as much inorganic phosphorus is liberated as lactic acid. But in a footnote to their 1928 paper Fiske and Subbarow state, "This is difficult to reconcile with the prevailing view that contraction is a response to increased acidity, but further investigation may show the inconsistency is only an apparent one."

If the hydrolysis of phosphocreatine is a mechanism for the neutralization of lactic acid should not there be a direct relation between the amount of phosphocreatine hydrolyzed and the amount of lactic acid produced? To test this hypothesis we have performed the following experiment.

Large frogs (Rana catesbiana) weighing 300 to 500 grams were quickly killed by cutting off their heads, their spinal cords were destroyed, and the sciatic nerves cut so that the gastrocnemii should be in as nearly a state of rest as possible. There was often some twitching, however, and effect of this is to be seen in the higher lactic acid content of the supposedly resting muscle which had to lie in the body for approximately half an hour until the manipulations on its fellow were completed. In the table the muscle left in the body for half an hour before being exercised is marked with an asterisk.

One-third of each exercised muscle was split off with sharp scissors, quickly thrown into liquid air, and ground to a powder. One-half of this powder

was treated with ice cold 8 per cent trichloracetic acid and kept at 0° to obtain a protein free filtrate for estimation of phosphocreatine, according to the method of Fiske and Subbarow (4), the other half was used for estimation of lactic acid by the Friedman, Cotonio, Shaffer method (5).

TABLE 1

Hydrolysis of phosphocreatine and lactic acid production in frog muscle

Phosphorus in mgm. per 100 grams. Lactic acid in per cent

RESTING MUSCLE						EXERCISED MUSCLE					
		Inorganic phosphorus + labile phosphorus	Inorganic phosphorus	Phosphocreatine	Lactic acid			Inorganic phosphorus + labile phosphorus	Inorganic phosphorus	Phosphocreatine	Lactic acid
4/12/29		41.3	20.5	20.8	0.088						
4/17/29	1st third	32.9	19.9	13.0	0.085	1st*	third resting	41.0	20.9	20.1	0.084
	2nd third	41.0	23.8	17.2	0.100	2nd	third worked	34.5	21.7	12.8	0.127
	3rd third	38.7	22.0	16.7	0.133	3rd	third worked	33.7	26.9	6.8	0.198
4/18/29	1st third	42.0	19.2	22.8	0.049	1st*	third resting	44.6	20.1	24.5	
	2nd third	44.0	22.2	21.8	0.070	2nd	third worked	34.7	25.2	12.2	0.181
	3rd third	41.7	19.0	22.7	0.102	3rd	third worked	38.1	24.9	13.2	0.185
						4/19/29	1st third resting	34.4	14.1	20.3	0.064
							2nd third worked	29.1	17.5	11.6	0.160
							3rd third worked	25.0	18.2	6.8	0.215
						4/19/29*	1st third resting	35.5	17.5	18.0	0.076
							2nd third worked	34.7	23.0	11.7	0.185
							3rd third worked	31.5	24.7	6.8	0.232
4/23/29	1st third	42.2	28.8	13.4	0.097	1st*	third resting	39.9	27.0	12.9	0.117
	2nd third	41.8	25.7	16.1	0.134	2nd	third worked	35.9	24.8	11.1	0.196
	3rd third	40.1	26.7	13.4	0.140	3rd	third worked	39.8	37.7	2.1	0.295
4/27/29	1st third	45.9	27.3	18.6	0.040	1st*	third resting	40.2	22.9	17.3	0.155
	2nd third	45.6	28.0	17.6	0.132	2nd	third worked	40.0	28.8	11.2	0.181
	3rd third	41.8	26.9	14.9	0.150	3rd	third worked	38.3	33.5	4.8	0.202

* Remaining in body ½ hour with sciatic nerve cut.

In four muscles a second third was removed and frozen after 8 minutes, and the remaining third after another 8 minutes, the muscle lying meanwhile on a glass plate at room temperature. In six muscles, after the first third had been removed the remainder was stimulated with single break shocks at 3 second intervals for 8 minutes. The muscle was then split in

two; one portion was frozen at once, and the remainder was stimulated for another 8 minutes before being frozen. The results are given in the accompanying table.

It will be seen that in the muscles which were not stimulated the phosphocreatine remained fairly constant, falling at most only 20 per cent, while the lactic acid was doubled and in one case nearly trebled. In the stimulated muscles the phosphocreatine content of the successive slices decreased and the lactic acid increased, but by no means proportionately. Since the lactic acid maximum for complete fatigue is 0.4 to 0.5 per cent we may consider that in our experiment the muscles were moderately exercised, since in only one case did the lactic acid content rise above 0.23 per cent. This concentration, 0.23 per cent, is the amount of lactic acid which Fiske and Subbarow estimate can be neutralized by the hydrolysis of the amount of phosphocreatine to be found in resting cat muscle, i.e., 0.45 per cent, corresponding to 60 to 70 mgm. inorganic phosphorus per 100 grams of muscle. But the average of the amounts of labile phosphorus found in eleven of our resting frog muscles was only 19.6 mgm. per 100 grams of muscle. This means that there is in frog's muscle approximately 0.14 per cent of phosphocreatine, about one-third that found in the cat. Therefore the amount of phosphocreatine to be found in the resting frog muscle should upon hydrolysis be able to neutralize a concentration of approximately 0.08 per cent lactic acid. It will be seen from the table that much more lactic acid than this can be formed and yet considerable amounts of the labile phosphorus compound be left. For example, on 4/19/29 there is an increase of lactic acid of 0.096 per cent after the first period of stimulation, yet only half the labile phosphorus disappears, and even after the lactic acid has been increased to 0.215 per cent, there remains a third of the original "resting" labile phosphorus.

These results show 1, that production of lactic acid from a cut surface of frog muscle causes little or in some cases no decrease in labile phosphorus; 2, although labile phosphorus disappears during muscular contraction the amount hydrolyzed by no means keeps pace with the lactic acid produced. There appears, therefore, to be no direct quantitative relation between hydrolysis of phosphocreatine and production of lactic acid.

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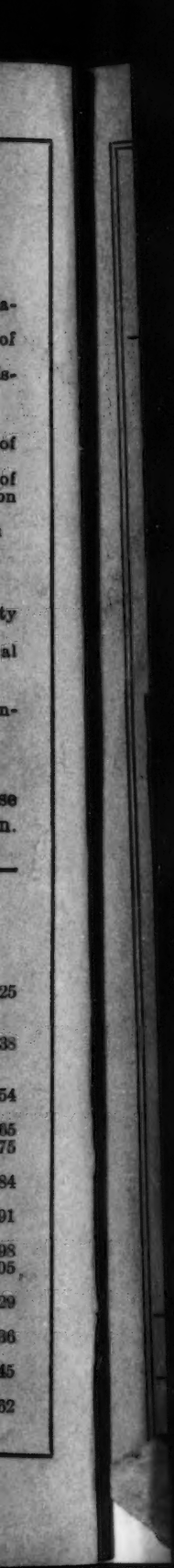
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